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THE ACTION OF ADRENALIN AND ACETYL-CHOLIN ON THE CORONARY ARTERIES OF THE RABBIT

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In the present investigation the effects of adrenalin and acetyl-cholin on the coronary flow of the perfused heart of the rabbit were studied because of the effects which these drugs are said to have on the sympathetic and vagus nerves. This work is intended as a preliminary to the study of the action of these nerves on the coronary arteries of the mammal.

Adrenalin has been extensively employed in studying the action of the sympathetic nerves on the coronary arteries of the mammal.[1] In those instances in which the action of the drug was investigated by the effect produced on the coronary circulation, the rate of flow was usually increased. The dose of the drug used was invariably sufficient to produce a striking stimulating action on the heart. This feature was considered by some to be responsible for the change in rate of coronary flow.

Brodie and Cullis (2) avoided the marked stimulating effect of adrenalin by reducing the size of the dose. In those instances in which minute quantities were employed, a decrease in rate of perfusion was observed. As the size of the dose was increased, causing the stimulating action to become more prominent, the decrease in the rate of perfusion was replaced by an augmentation. This is the only series of experiments to our knowledge in which it has been shown that opposing effects of adrenalin on the coronary arteries may be obtained by varying the size of the dose employed.

More recently, Drury and Smith (1) have studied, by means of the microscope, the effect of adrenalin applied locally to the coronary arteries of the tortoise. In all instances the vessel under observation was constricted. Sometimes the lumen was even entirely obliterated. After the vessels were constricted by adrenalin, they were then dilated to their

[1] For a review of the literature on the subject, the reader is referred to the article by Drury and Smith (1).

original size by faradic stimulation of the vagus. The dilating action of the vagus was readily eliminated by atropin.

Drury and Sumbal (3), in a later investigation, obtained a striking decrease in the rate of coronary flow of the perfused tortoise heart following the introduction of adrenalin into the perfusate. In the same series of experiments similar results were obtained by the stimulation of the sympathetic nerves.

EXPERIMENTAL. The heart was perfused at a pressure of 55 mm. Hg with a Locke-Ringer solution saturated with oxygen. The perfusate was kept at a constant temperature of 38°C. The rate of coronary flow was recorded by the tipping bucket method. A uniform cardiac rate was maintained by rhythmically induced break shocks in some experiments. The rhythmical stimulation was produced by an oscillating contact maker, employing the principle of the tuning fork, which was connected with the ordinary induction coil. This method has been described more in detail in a previous study (4).

Adrenalin. Adrenalin was introduced into the perfusate by injecting it with a hypodermic syringe directly into the tubing at its attachment to the aortic cannula. The concentration of adrenalin used was 1–10,000,000, 0.2 cc. of this being injected at a rate which would produce a dilution of approximately 1–200,000,000 in the coronary arteries. The above low concentration was employed to avoid the complicating effects produced by the extreme cardiac stimulation resulting from the higher concentrations which are ordinarily introduced. Adrenalin in this dilution produced a definite decrease in the rate of coronary flow varying from 12 to 22.5 per cent (fig. 1). The diminished perfusion rate began immediately after the introduction of the drug and persisted up to the point of greatest cardiac stimulation. At this point the coronary flow was usually slightly augmented for a short time (fig. 2a). Even with these minute doses of adrenalin there was a very distinct stimulating effect on the heart, as indicated by the acceleration in rate and increase in amplitude of contraction. In those experiments in which a uniform cardiac rate was maintained by rhythmical stimulation, the same action of adrenalin was observed (fig. 2b).

The above results demonstrate that adrenalin constricts the coronary arteries of the rabbit, as is shown by a decrease in the rate of coronary flow when the dose employed is sufficiently small to avoid extreme cardiac stimulation. It may be recalled that the decrease in rate of perfusion persisted to the point of maximum cardiac stimulation. Here the increase in rate and amplitude of cardiac contraction was apparently sufficient to overcome the constricting effect. It is felt that the latter feature has, in a large measure been responsible for the results heretofore reported.

The decrease in the rate of coronary flow of the tortoise obtained by Drury and Sumbal, following the introduction of adrenalin into the per-

fusate was greater than that obtained by us in the rabbit. It is possible that the slow cardiac rate of the tortoise and the absence of the marked stimulating action of adrenalin on the heart of the animal may explain the difference. The latter factor enabled these investigators to use concentrations of adrenalin in the tortoise which, if employed in the rabbit, would have produced a stimulating action on the heart sufficient to over-shadow any constriction of the coronary arteries.

Acetyl-cholin. Acetyl-cholin is generally recognized as having an inhibitory action on the heart comparable to that produced by stimulation of the vagus (5), (6), (7). The rate is reduced, and in some instances a

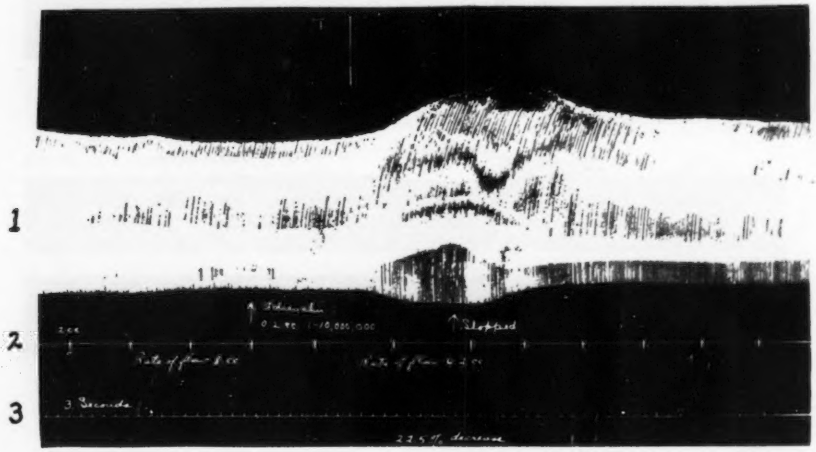

Fig. 1. The effect of adrenalin on the coronary flow. *1*, cardiac contractions; *2*, perfusion record each registration representing 2 cc.; *3*, time record at 3-second intervals.

During the period indicated by arrows adrenalin hydrochloride 1–10,000,000 dilution was administered at a rate which gave approximately 1–300,000,000 concentration in the coronary arteries. The perfusion rate was diminished 22.5 per cent.

transient cardiac arrest has been observed. Coincident with the reduction in rate, the amplitude of contraction may be diminished. In some experiments the diminution in the amplitude of contraction is the most striking feature. These effects on the heart may be greatly reduced or eliminated by atropin (7).

When acetyl-cholin is introduced into the circulation there is a decrease in the blood pressure which was first thought to be solely dependent on the cardiac effect. Dale (6) later showed, however, that even minute doses which produced no effect on the heart still gave a reduction in blood pressure. The drug is thus regarded as having an independent dilating action

on the vessel. In all instances in which a dilating action was observed by Hunt (7) the effect was diminished or prevented by atropin. Dale and Richards (8), in the study of histamine, employed acetyl-cholin as a control. They were inclined to believe, from this investigation, that the dilating effect of acetyl-cholin on the vessels was primarily due to the direct action of the drug on the smooth muscle of the vessel wall.

Very few observations have been made on the action of acetyl-cholin on the coronary arteries. Eppinger and Hess (9) reported a constriction

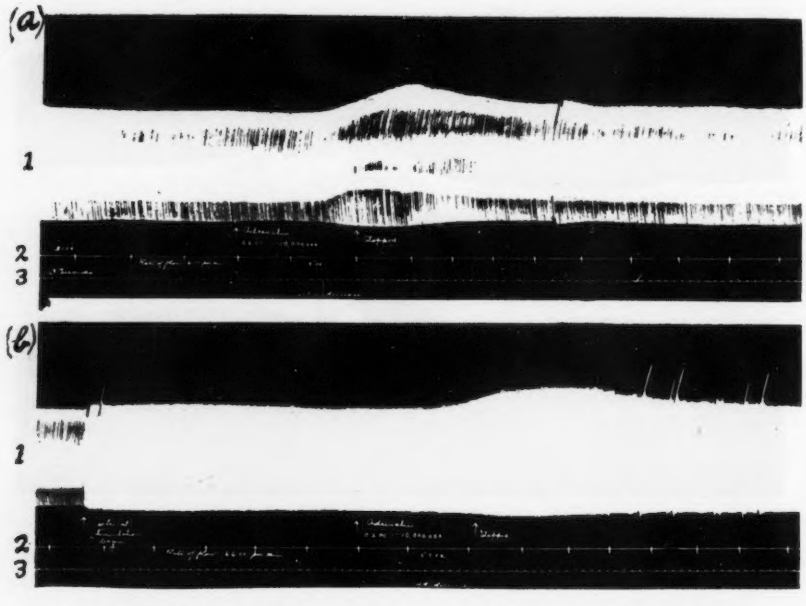

Fig. 2. The effect of adrenalin on the coronary flow. *a*, in this experiment the decrease in the rate of coronary flow was followed by an augmentation which began at the point of maximum cardiac stimulation. *b*, shows similar decrease in the rate of perfusion in an experiment in which the cardiac rate was controlled.

of isolated coronary strips. Sumbal (10) has more recently studied the effects of acetyl-cholin on the coronary arteries of the tortoise by direct application and perfusion methods. In the former, the drug was applied locally to an artery and the effects studied by microscope. A dilatation was observed. When the drug was introduced into the perfusate, an increase in the rate of coronary flow followed. In each instance the effect was completely abolished by atropin. Because of the similarity of the results to those produced by vagus stimulation, Sumbal considered that

they were dependent on the direct action of the drug on the vagus nerve endings.

In the present investigation acetyl-cholin was introduced into the coronary arteries in dilutions varying from 1–100,000 to 200,000. The drug in these concentrations greatly reduced the heart rate and in some instances produced cardiac standstill. Coincident with the reduction in rate, the amplitude of the contraction was diminished. With the onset of the above changes in the heart the perfusion rate was greatly augmented. This

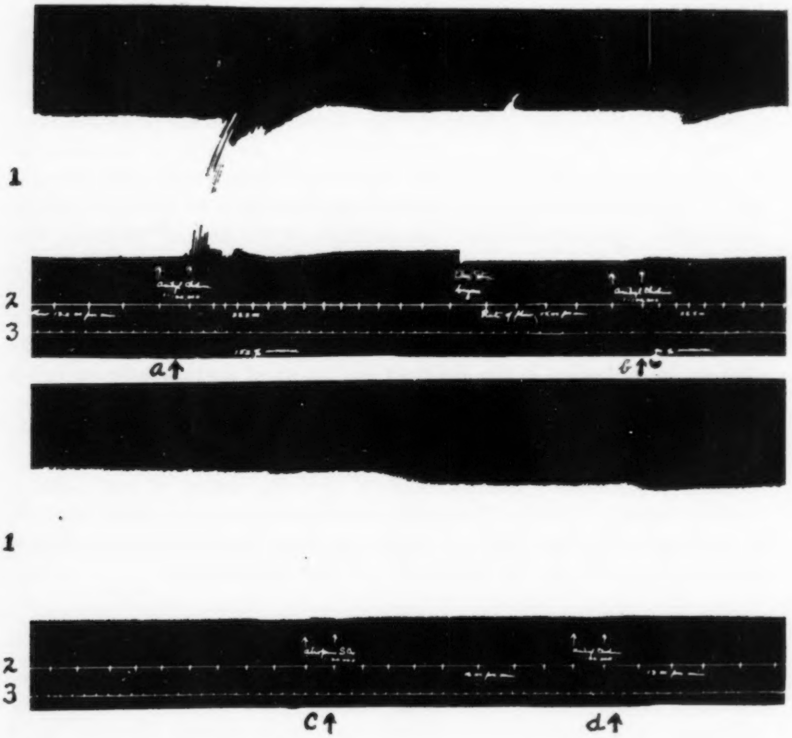

Fig. 3. Acetyl-cholin. The two tracings represent one continuous experiment. *a*, shows the effect of acetyl-cholin on rate and amplitude of cardiac contraction and on the coronary flow. *b*, shows the effect of acetyl-cholin in the same concentration when the heart rate is controlled by rhythmical stimulation. It is to be noted that there is a similar increase in the rate of perfusion. *c*, atropin introduced in concentration of 1–20,000. There is no change in the rate of coronary flow. *d*, acetyl-cholin was again administered in same concentration as *a* and *b*. The effects on the cardiac action and coronary flow were negligible.

increase, in some instances, amounted to 180 per cent. The slowing of the cardiac rate and the depression in amplitude of contraction were transient. The increase in rate of perfusion, however, persisted for some time after the above effects on the heart had subsided. The administration of atropin sulphate in dilutions of 1–20,000 eliminated the effects of subsequent doses of acetyl-cholin on the heart rate and in a large measure prevented the reduction in the amplitude of contraction. The atropin furthermore eliminated the action of the acetyl-cholin on the perfusion rate except for a very transient slight increase during the period in which there occurred the slight depression in amplitude of the contraction. In the earlier experiments in which higher concentrations of acetyl-cholin were employed, the depressing action of the drug on the amplitude of the contraction was more striking and prolonged. In these instances this feature was not particularly altered by atropin and the acceleration in the perfusion rate, even though greatly reduced, was still evident. A number of experiments were done in which the cardiac rate was controlled by rhythmical stimulation. This measure largely eliminated the depression in the amplitude of contraction by acetyl-cholin. The increase in the rate of coronary flow was, however, similar to that seen in the undriven heart.

These results show that acetyl-cholin dilates the coronary arteries of the rabbit and that the dilating action may be abolished by atropin. In this connection it may be recalled that in all instances in which Hunt (7) observed a dilatation in the study of the action of acetyl-cholin on other arteries, the effect was diminished or prevented by atropin. The action of acetyl-cholin on the coronary arteries of the rabbit is thus comparable to the action observed on other arteries.

These experiments furthermore show that the action of acetyl-cholin on the coronary arteries of the mammalian heart is similar to that observed in the heart of the tortoise. In the latter, Sumbal concludes that the action is comparable to that obtained by vagal stimulation.

SUMMARY

Adrenalin in concentrations of 1–200,000,000 decreased the rate of coronary flow in the perfused heart of the rabbit. The decrease in the rate of perfusion, varying from 12 to 22.5 per cent, began shortly after the introduction of the drug and continued to the point of maximum cardiac stimulation. The constricting action of adrenalin was then apparently overcome by the increased activity of the heart. When larger concentrations were used, the decrease in the rate of coronary flow was entirely replaced by an augmentation. It is thus felt that the increase in the rate of coronary flow heretofore reported may be attributed to the extreme stimulating action on the heart by the dose employed.

Acetyl-cholin in concentrations of 1–100,000 and 1–200,000 has an action on the heart of the rabbit comparable to that of vagal stimulation. Coincident with this action on the heart, the rate of coronary flow is greatly increased. The introduction of atropin into the perfusate in concentrations of 1–20,000 prevented or eliminated these effects. These results are similar to those observed in the study of the action of acetyl-cholin on the coronary arteries of the tortoise and on other arteries in the mammal.

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THE UTERINE CONTRACTING POWER OF THE SPINAL FLUID AFTER THE ADMINISTRATION OF EXTRACTS FROM THE SEX GLANDS AND OTHER ORGANS

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About two years ago Dixon (1) reported the results of the injection of various organ extracts upon the secretory activity of the posterior lobe of the pituitary gland of the dog. In this investigation boiled saline extracts were given intravenously to tracheotomized dogs under anesthesia and the effect upon the pituitary gland studied by estimating the amount of the uterine contracting material in the spinal fluid. For this purpose a definite volume of spinal fluid was added to the surviving guinea-pig uterus suspended in Locke's solution, and its effect upon the contraction of the organ compared with that produced by a standard pituitrin solution. Of the extracts tried no effect was obtained from preparations of liver, brain, choroid plexus, testis, epididymis, anterior lobe pituitary gland, thyroid and pancreas. Certain chemical substances including histamine were likewise negative. Extracts from the interstitial portion of the ovary alone gave positive results, while corpus luteum extracts produced no effect. Two or three minutes after the injection of the interstitial extract, there was demonstrable in the spinal fluid a considerable increase in a smooth-muscle contracting principle supposedly coming from the posterior lobe of the pituitary gland. The effect was over in 10 to 15 minutes. From these results Dixon drew the conclusion that extracts from the interstitial tissue of the ovary specifically excite the pituitary gland to secrete.

Similar results were also obtained with extracts of the posterior lobe of the pituitary gland, but in this case a somewhat different interpretation was placed upon the observation from that used to explain the findings with ovarian extracts.

Although Dixon found, in conformity with the report of Cow, (2) that extracts made from the duodenal mucosa, also, produced such an augmentation of pituitary secretion, this, according to him, did not invalidate the conclusions with respect to the ovarian effect, because of apparently essential differences in the latent period, intensity, constancy and duration of action of the two preparations.

More recently Dixon and Marshall (3) found that ovaries containing corpora lutea, whether of pregnancy or of estrus, were without effect upon the pituitary secretion so long as the corpora lutea remained in a state of full activity. During the retrogressive stages of the corpora lutea the power of the ovary to stimulate the pituitary gland varied directly with the extent of involution reached by the yellow bodies. These findings led the authors to an attractive hypothesis as to the cause of the onset of parturition and to the inference that satisfactory results from ovarian therapy can be expected only if glands from animals at certain stages of pregnancy or of estrus are used.

To our knowledge no attempt has thus far been made to repeat these experiments. It seemed to us of considerable importance to confirm these findings. If it were proved that the effect of the ovary upon the pituitary gland is truly specific, as claimed by Dixon, this would establish an ovario-pituitary interrelationship of great physiological and pathological significance. Moreover, the work seems to offer possibilities for further research, as there are a number of problems that readily suggest themselves in connection with it.

Experimental. The work to be reported represents an effort to confirm the results of Dixon with regard to the effect of organ extracts upon pituitary secretion. The experimental procedure was essentially that of Dixon. At first the urethane-morphine-ether anesthesia recommended by this author was used. In connection with some later experiments we had expected to make certain observations upon the blood sugar level after the administration of organ extracts. For this reason amytal (Lilly) was chosen as the anesthetic because of its supposed non-effect upon the blood sugar level, as claimed by Paige (4). Unfortunately we found that amytal produced irregular fluctuations in the concentration of the blood sugar of the dog, rendering impossible any attempt to study this blood constituent under our experimental conditions. The results in relation to the oxytocic power of the spinal fluid were, however, unaffected by the change in the anesthetic. Since blood and inflammatory products may cause a contraction of the guinea pig uterus, care was taken to exclude these elements, as far as possible, from the samples of spinal fluid. In adopting a technique for the pituitrin assay due regard was paid to the recommendations made in this connection by Burn and Dale (5) and by Smith and McCloskey (6).

In order to avoid the excessive dilution of the oxytocic principle of the spinal fluid, the capacity of the muscle chamber was reduced from the customary 80 or 100 cc. to 35 cc. and the uterus was bathed in frequent changes of about 25 cc. of Locke's solution. The detection of minute amounts of oxytocic material was further facilitated by the employment of a long muscle lever, giving a magnification of about 1:6. The water bath was heated by means of an electric lamp, connected in series to an

efficient thermostat which kept the temperature constant to within at least 0.1°C.

Instead of presenting our results as per cent of pituitrin or as milligrams of fresh infundibular tissue, we have found it more convenient to express them in terms of per cent of maximum contraction produced in the uterus by the spinal fluid. Thus, if 2 cc. of spinal fluid from a dog during a control period evoked no contraction, while a like volume of spinal fluid from the same animal after receiving an extract caused a contraction of the uterine muscle one-half as great as the latter is capable of showing with pituitrin, the result was said to be 50 per cent and the extract presumably responsible for it designated as of corresponding activity. This method was resorted to because of the wide variability in the strength of the pituitrin solutions which we were obliged to use as a standard. The rationale for this mode of presentation may be found in the proportionality between the amount of pituitrin and the intensity of the contractile reactions produced. It is well known that fractional parts of a minimum amount of pituitrin required to produce maximum contractions of the uterine muscle will cause contractions in the same test object of proportionately reduced magnitudes.

Most of the extracts were made with glycerol. The glycerol was allowed to remain in contact with the hashed tissues for several days, after which it was filtered off and the residue washed with water, the washings being added to the glycerol extract. Cow ovaries obtained fresh from the slaughter house were used. The corpora lutea were removed from each gland as completely as possible and extracted separately from the remaining interstitial tissue.

More than 150 experiments were made on about 120 dogs. It may be stated at the outset that positive results were obtained with extracts of liver, spleen, testicle, corpus luteum and the interstitial tissue of the ovary, in addition to the dried whole ovary of commerce, and histamine. Furthermore, these substances were found to be effective when given by mouth to either male or female dogs or to female castrates. Extracts of kidney, pancreas, skeletal and heart muscle yielded negative results, as well as numerous control experiments with the anesthetic alone and with the solvents used in the preparation of the extracts.

Before proceeding to the discussion of these results a word may be said concerning the occurrence of a plain muscle stimulating substance in the spinal fluid. A brief review of the literature on this subject, giving the contradictory opinions of a number of investigators, was recently presented by Miura (7). This author confirmed the results of Trendelenberg (8) that the action of 1 cc. of spinal fluid on the surviving uterus corresponds to that of about 1/35,000 mgm. of fresh infundibular tissue. These results contradict the claim of Dixon that the oxytocic titre of 1 cc. of the spinal fluid of the dog may be as high as 2 mgm. of fresh posterior lobe of pituitary gland.

Disregarding these purely quantitative differences, from a qualitative standpoint it would appear that the spinal fluid of normal animals contains an oxytocic principle. The identity of this material with the internal secretion of the posterior lobe of the pituitary gland recently obtained by Abel, Rouiller and Geiling (9) in a highly active form, is still an open question.

The unqualified acceptance of the view of some authors that the hormone of the pituitary gland is discharged into the blood through the spinal fluid is, therefore, not justified. Moreover, while from an anatomical standpoint it is possible for the secretion of the posterior lobe to be poured into the spinal fluid, it is difficult to understand why the discharge of the hormone to the general circulation should be by way of this circuitous route in preference to the more direct one through the efferent vessels of the gland.[1]

Becht and Matill (10) have shown that the dura mater presents a serious barrier to the passage of some drugs from the spinal canal into the blood. Thus adrenalin or nicotine injected into the arachnoid spaces escape into the blood at such a slow rate that they fail to produce any systemic effect. It would be interesting to know whether or not this is true of pituitrin. Practically nothing is known concerning the rôle of this hormone in the spinal fluid. Nor is there any satisfactory explanation offered for the persistence of a uterine contracting substance in the spinal fluid after hypophysectomy as found by Dixon and as suggested by the experiments of Galowsky (mentioned by Fromherz (11)).

Dixon claimed that of the normal spinal fluid of dogs sometimes as little as one or two drops will cause a maximum contraction of the guinea pig uterus. In most cases this effect was obtained from 10 drops of liquid, indicating a pituitrin content of from 1 to 10 mgm. of fresh tissue. This does not agree with the earlier findings of Cow who stated that 3 cc. of dog's spinal fluid might evoke contraction and is wholly at variance with our own results. Of 138 samples of normal spinal fluid from dogs, ranging in quantity from 2 to 9 cc., we have found but two that gave positive results. In these instances 100 per cent contraction of the guinea-pig uterus was obtained from 2.6 and 2.4 cc. spinal fluid respectively, and the latter came from an oöphorectomized dog. These results obviously do not lead to the conclusion that an oxytocic substance in measurable quantity is a common constituent of the normal spinal fluid.

Effect of glycerol extract of the interstitial tissue of cow ovaries on the concentration of a pituitrin-like substance in the spinal fluid. Of eleven

[1] Since this paper was submitted for publication there has appeared a review of the literature on the pituitary body by Geiling (Physiological Reviews, January, 1926). In this paper the question of the mode of exit of the pituitary secretion from the gland is discussed in greater detail.

TABLE 1

Effect of glycerol extract of the interstitial tissue of cow ovaries

(5 cc. extract = 1 gram fresh tissue)

SEX	WEIGHT DOG	NORMAL SPINAL FLUID		VOLUME EXTRACT GIVEN	TIME AFTER EXTRACT	VOLUME SPINAL FLUID	CONTRACTION	REMARKS
	kgm.	*cc.*	*per cent contraction*	*cc.*	*minutes*	*cc.*	*per cent*	
♂	13	6.6	0	20	30	1.0	100	Intravenous administration
					60	1.0	100	
					120	0.3	100	
					180	0.3	100	
♀	7.5	3.8	0	10	30	2.55	0	Intravenous administration
					60	2.9	0	
					90	1.4	100	
♂	15	3.75	0	20	30	4.0	0	Intravenous administration
					60	2.5	100	
					90	2.5	100	
					120	3.0	100	
♂	8.1	2.1	0	25	10	2.5	0	Intravenous administration
					60	2.7	0	
					120	1.0	100	
					180	1.0	100	
♀	10	3.0	0	60	60	3.0	0	Oral administration
					120	2.0	100	
♂	9.3	2.9	0	60	60	2.9	0	Oral administration
					120	3.0	100	
					180	2.0	100	
♀	6.5	2.7	0	35 + 10	60	2.1	100	Oral administration
					180	2.4	0	
					60	2.2	100	
♀	10	2.0	0	50	60	2.0	25	Oral administration
					200	2.0	100	
					240	2.3	90	
♂	12.9	2.7	0	50	60	1.9	20	Oral administration
					180	2.0	100	
					285	2.0	100	
♀*	15.6	2.4	0	50	60	2.0	100	Oral administration
					180	2.8	40	

TABLE 1—*Concluded*

SEX	WEIGHT DOG	NORMAL SPINAL FLUID		VOLUME EXTRACT GIVEN	TIME AFTER EXTRACT	VOLUME SPINAL FLUID	CONTRACTION	REMARKS
	kgm.	*cc.*	*per cent contraction*	*cc.*	*minutes*	*cc.*	*per cent*	
♀	11.4	2.5	0	50	90	1.5	80	Oral administration
					180	1.0	100	
♀*	11.6	3.0	0	35	60	2.4	0	Oral administration
					180	2.4	40	
					300	1.6	100	

* Castrate.

experiments with the intravenous injection of this material, 9 gave positive results. The increase in the oxytocic material of the spinal fluid of 5 dogs was noted in from 1 to 1½ hours after injection. Earlier reactions were given by four dogs. One of these showed an effect in 3 minutes, another in 15 minutes, while the remaining two reacted in 30 and 40 minutes, respectively. The height of the reaction was reached in most cases in about 2 hours and lasted in some dogs for 3 hours. There seems to be, however, a wide individual variation in the response of the animals in a series.

It was recently found by Kubie and Shults (12) that the concentration of sugar and nitrogen in successive samples of spinal fluid was greatly increased as the flow of the fluid diminished. To what extent our results were influenced by the diminution in the amount of fluid in the cerebrospinal canal we cannot say. The negative results of numerous control experiments would suggest that this did not have a measurable effect on the concentration of the oxytocic material in the spinal fluid. However, in order to minimize the danger of error from this source, we have avoided taking the samples of spinal fluid at too frequent intervals.

Seventeen dogs received the extract by stomach tube, mostly in doses corresponding to 1 or 1.5 grams of fresh tissue per kilogram of body weight. The effect on the uterine contracting substance of the spinal fluid became evident in most cases in about 2 hours or slightly more; in exceptional cases in one hour. It lasted as a rule for about 5 hours and always disappeared over night. Thus it may be seen that the effect by the two modes of administration is essentially the same, except as to minor differences in the latent period and duration of action.

No hard and fast distinction, however, can be drawn on these grounds, as there seems to be a number of factors influencing the results. Among these may be counted the responsiveness of the animal to the extract, the dose of the latter and probably numerous others not so evident. As an illustration the following experiment may be cited. A 14-kgm. dog failed

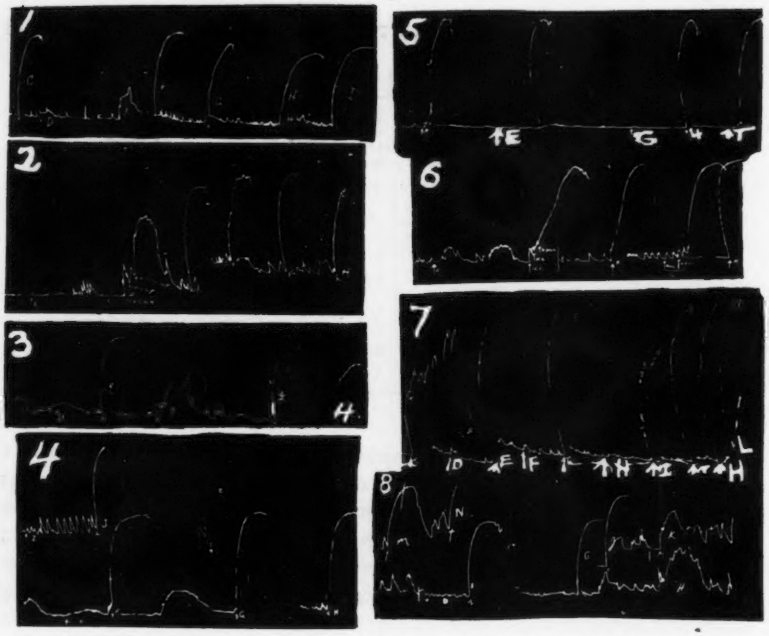

Fig. 1. Oral administration of glycerol extract interstitial tissue cow ovary to male dog. *C. F, I,* pituitrin curves. *D,* 2.7 cc. normal spinal fluid. *E,* 1.9 cc. spinal fluid one hour after extract. *G,* 2.0 cc. spinal fluid 3-hour sample. *H,* 2.0 cc. spinal fluid 5-hour sample.

Fig. 2. Glycerol extract corpus luteum cow ovary by mouth to female dog. *C,* 2.75 cc. normal spinal fluid. *D,* 2.75 cc. spinal fluid 1 hour after extract. *E, G,* pituitrin curves. *F,* 1.6 cc. spinal fluid 3 hours after extract. *H,* 1.4 cc. spinal fluid 3-hour sample, same as *F.*

Fig. 3. Glycerol extract corpus luteum cow ovary injected into vein of male dog. *C,* 2.6 cc. normal spinal fluid. *D,* 2.6 cc. spinal fluid 10 minutes after extract. *E, G,* pituitrin curves. *F,* 2.1 cc. spinal fluid 1-hour sample. *H,* 2.3 cc. spinal fluid 2 hours after injection of extract.

Fig. 4. Oral administration of glycerol extract of pig testicle to female dog. *E,* 2.2 cc. spinal fluid normal. *F, J,* pituitrin curves. *G,* 2.0 cc. spinal fluid 3 hours after feeding extract. *H,* 0.85 cc. spinal fluid 3-hour sample, same as *G.* *I,* 2.6 cc. spinal fluid 1 hour after extract.

Fig. 5. Glycerol extract of liver given by mouth to female dog. *D,* 2.7 cc. spinal fluid 3 hours after feeding extract. *E,* 2.5 cc. normal spinal fluid. *F, H,* pituitrin curves. *G,* 1.9 cc. spinal fluid 1-hour sample. *I,* 2.0 cc. spinal fluid 5-hour sample.

Fig. 6. Glycerol extract spleen fed to female dog. *D,* 2.5 cc. spinal fluid normal. *E,* 2.3 cc. spinal fluid 1-hour after extract. *F, G,* 1.0 cc. spinal fluid 3-hour sample. *H,* pituitrin solution.

Fig. 7. Histamin by mouth to male dog. *C, E, G, J, L,* pituitrin curves. *D,* 2.2 cc. normal spinal fluid. *F,* 1.4 cc. spinal fluid one-half hour after 20.0 mgm. histamin. *H,* 2.2 cc. spinal fluid, 1-hour sample. *J,* 2.0 cc. spinal fluid 3-hour sample. *K,* 1.0 cc. spinal fluid 3-hour sample, same as *J.*

Fig. 8. Intravenous injection of follicular fluid to male dog. *D,* 2.1 cc. normal spinal fluid. *E, G, J, N,* pituitrin curves. *F,* 2.1 cc. spinal fluid 15 minutes after giving 15 cc. crude follicular fluid from cow ovaries. *H,* 2.1 cc. spinal fluid 1 hour 15 minutes after injection. *K,* 2.2 cc. spinal fluid 1 hour after a second injection of 15 cc. follicular fluid. *M,* 2.2 cc. spinal fluid 2 hours after second dose follicular fluid.

to show any effect in $1\frac{1}{2}$ hours with 3 cc. of a commercial extract (Parke, Davis' ovarian residue, 1 cc. = 1 gram) given intravenously, but did respond in $1\frac{1}{4}$ hours to a second dose of 3 cc. injected $1\frac{1}{2}$ hours after the first. Five cubic centimeters of the same material were then given orally to a 7.0-kgm. dog, producing an effect in $3\frac{1}{2}$ hours, whereas a 5.5-kgm. dog responded to a like administration of 8 cc. in 1 hour and 20 minutes.

Results essentially similar to the above were obtained with commercial dried ovarian powder (Armour) and an extract made as described by Dixon.

Comparing our results with intravenous injection of interstitial extracts with those of Dixon it may be seen that the latent period in our experiments is much longer and the effect more prolonged. In these respects our findings agree more closely with those contained in the more recent report of Dixon published with Marshall. These authors call attention to the discrepancy but do not account for it satisfactorily. Furthermore, we cannot fail to notice that our results are not materially different from those obtained by Cow with duodenal extracts.

In discussing the work of Cow, Dixon stated that the effect of duodenal extract upon the pituitary gland differed from that of the ovarian extract as to latent period and constancy of action and based his claim for the specificity of the ovarian effect upon this alleged difference. This deduction is not supported by the results of our own work, nor is it borne out by the data published in a later paper by Dixon and Marshall in which no mention is made of the work of Cow.

Since there seemed to be strong reasons for questioning the specificity of the stimulating effect of the interstitial tissue of the ovary upon the pituitary gland, experiments were undertaken with extracts made from the corpora lutea and other organs.

Effect of corpus luteum extract. Experiments with this material did not yield consistent results. Several of the results had to be discarded because of bloody spinal fluid or poor condition of the guinea pig muscle. The intravenous administration of a glycerol extract to one male dog produced a positive result in 60 minutes which became more marked after two hours. Parke Davis' corpus luteum extract was negative. This agrees with the report of Dixon on this extract. Watery extracts of corpora lutea made by a method similar to Dixon's yielded negative results. The lipoid fraction of these bodies extracted with alcohol and taken up in corn oil according to the method of Doisy, Ralls, Allen and Johnston (13) was likewise negative. The oral administration of glycerol extract of corpus luteum to two female dogs produced an effect in from 1 to 3 hours. Two other females similarly treated, one a castrate, failed to respond.

The evidence from such a small series of dogs cannot be deemed final. It seems to suggest, however, that glycerol extract of corpora lutea may

produce a positive effect in some dogs. Whether these irregular results are due to the admixture of interstitial tissue in varying amounts or to some other factor, we cannot at present say. It should be recalled in this connection that the interstitial cells take some part in the formation of the corpus luteum and that a complete mechanical separation of the two anatomical elements is not altogether possible.

No attempt was made to classify the corpora lutea according to their developmental stages. This may have a bearing upon the results for, as

TABLE 2

Effect of glycerol extract of corpus luteum

(5 cc. extract = 1 gram fresh tissue)

SEX	WEIGHT DOG	NORMAL SPINAL FLUID		VOLUME EXTRACT GIVEN	TIME AFTER EXTRACT	VOLUME SPINAL FLUID	CONTRACTION	REMARKS
	kgm.	*cc.*	*per cent contraction*	*cc.*	*minutes*	*cc.*	*per cent*	
♂	13.2	2.6	0	30	15	2.6	0	Intravenous administration
					60	2.1	50	
					120	2.3	100	
♀	6.3	4.0	0	50	60	2.2	0	Oral administration
					180	2.2	80	
					300	2.0	100	
♀	11.8	2.75	0	50	60	2.75	80	Oral administration
					180	1.6	100	
					180	1.4	100	
♀*	15.6	2.1	0	50	60	2.2	0	Oral administration
					180	2.2	0	
♀	10.2	2.5	0	50	60	2.5	0	Oral administration
					180	1.9	0	

* Castrate.

Zondek and Aschheim (14) have shown, the intramuscular implantation of segments of corpus luteum, in certain stages, will bring on estrus in a castrated mouse. At other times the corpus luteum cannot exert such an influence. Frank and Gustavson (15) found that involuting corpora lutea most regularly furnished an estrogenetic hormone, while other forms less frequently and in much smaller amounts. Allen and Doisy (16) have shown that the follicular hormone which these authors demonstrated to be the causative mechanism in the phenomenon of estrus, can be extracted from the human corpus luteum in the third month of pregnancy.

These findings also give evidence of the fact that certain physiological properties are common to both the interstitial tissue and the corpus luteum. In addition Papanicolaou (17) showed that water extracts derived from either component of the ovary have a stimulating effect upon this gland and cause a shortening of the diestrous period in the guinea pig. It might not be illogical to suppose that these two extracts would not differ in their power to augment the oxytocic value of the spinal fluid. The foregoing considerations, together with the evidence which we have obtained with

TABLE 3
Effect of glycerol extract of testicle
(5 cc. = 1 gram fresh tissue)

SEX	WEIGHT DOG	NORMAL SPINAL FLUID		VOLUME EXTRACT GIVEN	TIME AFTER EXTRACT	VOLUME SPINAL FLUID	CONTRACTION	REMARKS
	kgm.	*cc.*	*per cent contraction*	*cc.*	*minutes*	*cc.*	*per cent*	
♀	5	4.8	0	15	15	3.1	0	Intravenous administration
					45	3.2	100	
					135	4.0	100	
					195	3.6	80	
♂	10	3.4	0	50	60	3.0	0	Oral administration
					180	2.7	0	
					240	1.0	100	
					285	1.0	100	
♂	9.6	2.8	0	50	120	2.8	100	Oral administration
					180	2.8	100	
					240	1.7	100	
					300	2.0	100	
♀	13.0			50	60	2.6	0	Oral administration
					180	0.85	100	
♂	7.5	2.2	0	35	60	2.3	0	Oral administration
					180	2.0	50	
					300	2.5	100	

glycerol extract of corpus luteum, make it difficult to accept the conclusion of Dixon that corpus luteum extracts are consistently without effect upon the uterine contracting substance in the spinal fluid. It seems advisable, however, to reserve judgment in the matter pending further investigation.

Effect of glycerol extract of testicle. One female dog was given an intravenous injection of glycerol extract of pig testicle, yielding results practically identical with those from interstitial extracts of the ovary. Neither do the results with mouth feeding show any material deviation from those obtained with ovarian feeding.

In addition to the cases tabulated we have a number of experiments with positive results which were omitted from consideration because of slightly bloody spinal fluids. These could be drawn upon for additional evidence in favor of our conclusion with respect to testicular extracts. In the first place the samples were centrifuged and the clear supernatant liquid used; secondly, we know from experience that such traces of blood serum alone

TABLE 4

Effect of oral administration of glycerol extract of liver

(5 cc. = 1 gram fresh tissue)

SEX	WEIGHT DOG	NORMAL SPINAL FLNID		VOLUME EXTRACT GIVEN	TIME AFTER EXTRACT	VOLUME SPINAL FLUID	CONTRACTION
	kgm.	*cc.*	*per cent contraction*	*cc.*	*minutes*	*cc.*	*per cent*
♀	7.5	2.5	0	35	90	2.1	0
					180	2.0	90
♀	7.2	2.7	0	35	60	3.0	30
					180	2.6	50
					240	2.0	80
♀	9	3.5	0	35	60	2.0	100
					180	1.5	100
♀	9	2.8	0	50	90	2.0	70
					180	2.0	90
♂	10.2	2.5	0	50	60	2.6	100
					180	0.7	100
♀	10	3.1	0	50	60	2.2	70
					180	1.1	100
♀	9	2.9	0	40	60	2.2	0
					180	2.0	100
					300	2.2	50
♂	11.8	2.5	0	50	60	1.9	0
					180	2.4	100
					300	2.0	100

could not have had the oxytocic effect shown by the spinal fluids containing them. But even without this evidence there seems to be but very little doubt that the influence of testicle upon the oxytocic titre of the spinal fluid is not much different from that exhibited by ovarian extracts. Cow found orchitic extracts negative.

Effect of glycerol extracts of liver and of spleen. Thirteen dogs were fed extract of liver. Ten showed positive results.

Four dogs received an extract of spleen. Both intravenous injection and mouth feeding seem to cause a marked increase in the uterine contracting power of the spinal fluid.

Effect of histamine acid phosphate. A study of our data with glycerol extracts of various organs producing positive results reveals with striking emphasis the similarity in the mode of action of the different preparations.

TABLE 5

Effect of glycerol extract of spleen

(5 cc. = 1 gram fresh tissue)

SEX	WEIGHT DOG	NORMAL SPINAL FLUID		VOLUME EXTRACT GIVEN	TIME AFTER EXTRACT	VOLUME SPINAL FLUID	CONTRACTION	REMARKS
	kgm.	*cc.*	*per cent contraction*	*cc.*	*minutes*	*cc.*	*per cent*	
♀	11.8	2.5	0	50	60	2.3	100	Extract given orally
					180	1.0	100	
♀	7	2.0	0	35	60	2.0	0	Extract given orally
					180	0.7	100	
					300	1.6	100	
♀	7	2.2	0	15	5	2.1	0	Extract given in vein
					30	2.0	0	
					90	1.8	40	
					180	2.0	100	
♀	9.4	5.6	0	25	60	2.0	0	Extract given in vein
					180	2.4	100	
					300	3 0	0	

This seems to point to a single substance which is probably present in all the extracts and to which this similarity of effect is due.

Histamine naturally suggests itself, even though Dixon obtained no effect in ½ hour from the injection of 20 mgm. of this substance into the vein of a dog. We found, on the contrary, that feeding histamine in doses of about 1.0 mgm. or more per kilogram of body weight gave uniformly positive results in 9 dogs. The character of the response was very similar in most particulars to that produced by the organ extracts.

This result seems at first somewhat unexpected, in view of the claim of Koessler and Hanke (18) that histamine is rendered pharmacologically inert in its passage through the intestinal wall. They state that the ingestion of as much as 500 mgm. produces no symptoms in a 5-kgm. dog. Since it was found that more than one-half of this amount was absorbed, the histamine must have become detoxicated. Granting the correctness

of this explanation, the possibility of this altered form of histamine entering the general circulation still remains and the fact that it no longer causes a fall of blood pressure or dyspnea does not necessarily prove that it has also lost its oxytocic activity or that it cannot augment the uterine stimulating

TABLE 6

Effect of histamine acid phosphate given orally

SEX	WEIGHT DOG	NORMAL SPINAL FLUID		HISTAMINE	TIME AFTER EXTRACT	VOLUME SPINAL FLUID	CONTRACTION
	kgm.	*cc.*	*per cent contraction*	*mgm.*	*minutes*	*cc.*	*per cent*
♀	13.2	2.2	0	200.0	60	1.7	100
♀	13.2	2.1	0	20.0	30	1.95	0
					60	2.0	80
					180	2.0	90
					240	4.0	10
♀	9.0	2.0	0	10.0	60	2.0	0
					180	2.0	60
					300	2.0	100
♂	9.7	2.2	0	20.0	30	1.4	0
					60	2.2	0
					180	1.0	100
♂	15	2.1	0	20.0	60	1.7	75
					180	2.1	25
					240	2.1	10
♀	16	2.7	0	20.0	60	2.5	0
					180	2.6	75
					300	2.6	90
♀	9.4	2.7	0	20.0	180	2.7	80
					270	2.4	50
♀	9.4	3.8	0	20.0	60	2.3	0
					180	2.5	25
					270	2.5	80
♀	9.8	2.4	0	20.0	60	2.1	0
					180	2.0	100

capacity of the spinal fluid. Whether histamine produces this latter result by a mechanism other than its direct entrance into the spinal fluid from the blood, we cannot definitely say now. It should not be difficult to decide this point and we hope to report on it in the near future.

The close agreement between the results with organ extracts and with

histamine naturally raises the question as to whether the effect of the former is not due to the presence of the latter. We have attempted, therefore, to determine the histamine content of our extracts by the method of Hanke and Koessler (19). This work did not lead to satisfactory results, because of certain difficulties in the technique when applied to our material. We believe, however, that the probable amounts of histamine which might have been present in the extracts administered could have been but very small and it remains to be seen whether such quantities can exert an effect on the spinal fluid. In connection with the foregoing, account should be taken of the fact that extracts of kidney and pancreas as well as Witte's peptone and autolized blood given by mouth were without effect.

Since Dixon claimed that the increase in the concentration of the oxytocic material in the spinal fluid is due to the stimulation of the posterior lobe of the pituitary gland by the ovarian extract, and since Dixon and Marshall claimed that this pituitary exciting power of the ovary of the non-pregnant animal is greatest during estrus; it seemed of interest to know whether this effect is associated with the lipoid hormone obtained by Doisey, Ralls, Allen and Johnston (13) from the follicular fluid, ovary and placenta. Allen and Doisy (20) showed that extracts derived from these sources are capable of inducing estrous-like manifestations in the genital tract of the spayed mouse. Papanicolaou and Blau (21) have demonstrated the occurrence of a similar substance in ovarian cystic fluid. Loewe (22) succeeded in extracting an estrogenetic hormone from the blood of sexually mature females. This was also accomplished independently by Frank, Frank, Gustavson and Weyerts (23).

It seems thus fairly well established that estrus is caused by a definite hormone which is brought into play at certain periods during the sex-life of the female animal. Whether this hormone is directly responsible for any other of the diverse phenomena which characterize ovarian function is not known. There seemed to be some ground for the belief that it might be a factor in the pituitary reaction described by Dixon, in view of the fact that, unlike Dixon and Marshall, we have obtained a positive effect from the injection of follicular fluid into the vein of a dog. On the other hand, some doubt is cast on the possibility of such a relationship by the fact that liver, testicle and spleen gave positive results.

Experiments were therefore made with the lipoid and water-soluble fractions of the interstitial tissue of the ovary. It was found that the lipoids, whether given by mouth or in the vein, were never as effective in causing a spinal fluid reaction as the lipoid-free extracts. In one dog we determined approximately the minimum amount of watery extract corresponding to a given weight of fresh tissue which would evoke a 100 per cent response in 3 hours. This was found to be about 200 mgm. per kgm. of body weight, given orally. The same dog gave only a doubtful

reaction at the end of 5 hours after receiving four times the amount of lipoid extract by stomach tube. In another dog 3.33 grams of lipoid extract per kilogram of body weight given orally produced a 100 per cent reaction in 3 hours. This comparison would be more convincing had the extracts been given intravenously. As it stands, it may merely indicate a difference in the rate of absorption between the two preparations. Nevertheless, since even the intravenous injection of the lipoids was, as a rule, less effective than the oral administration of the watery extract, it seems justifiable to assume that the effect of the ovarian extract on the spinal fluid is due mainly to some water-soluble constituent. Furthermore it was shown by one of us (N. F. B.) that the Parke Davis extract of "ovarian residue" which proved highly effective in causing a spinal fluid reaction, was powerless to bring about any changes in the estrous cycle of three guinea pigs.

Attention may now be called to an observation which we have made during the course of this work; namely, that the results upon bulldogs were always negative no matter what extract was used or how it was given. This was also true of the oral administration of histamine. While occasionally dogs of other breeds failed to react to a treatment which gave positive results in most animals, with this breed the failure to respond to any treatment was so consistent that we are inclined to regard it as a racial rather than an individual peculiarity.

The results detailed above have an obvious bearing upon the hypothesis of Dixon and Marshall that an ovario-pituitary mechanism under the control of the corpus luteum is an important factor in the onset of labor. However, we do not wish to stress their significance in this connection now, because of the incompleteness of our evidence. It might be pointed out, however, that the phenomena associated with parturition may perhaps be more satisfactorily accounted for by the experimental results of Brdiczka (24). This investigator observed a strongly oxytocic substance in the sera of pregnant women near term and during labor which apparently came from the placenta and was not of endocrine origin.

SUMMARY

1. Normal spinal fluids of dogs, in quantities of from 1 to 9 cc., but rarely cause a contraction of the surviving guinea-pig uterus, indicating the probable absence of an oxytocic principle in this fluid.

2. After the administration of certain organ extracts to male or female dogs or female castrates, either by vein or orally, it becomes possible to demonstrate an oxytocic substance in relatively small amounts of spinal fluid.

3. The following proved effective in this regard: glycerol, watery and lipoid extracts of the interstitial tissue of the ovary, glycerol extracts of

testicle, liver and spleen and a commercial ovarian extract (Parke Davis Ovarian Residue).

4. Equally positive results were obtained from the oral administration of dried ovarian powder (Armour) and of histamine.

5. The mode of action of these various substances upon the dogs was similar in each case.

6. Glycerol extract from corpora lutea gave inconclusive results in a small series of dogs.

7. The effect of the fat-soluble extracts of the interstitial tissue of the ovary was less marked and more inconstant than that of the water-soluble extracts.

8. No increase in the uterine contracting activity of the spinal fluid was observed after giving lipoid extracts of corpus luteum, glycerol extract of kidney, pancreas, skeletal and heart muscle and a mixture of glycerol and autolized blood.

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ON THE SOURCE OF VITAMIN B IN NATURE[1]

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It has already been pointed out by one of us (Burrows, 1915) that growth, differentiation and function as they occur in the cells of the normal organism are not changes peculiar to the ageing cells, but the immediate result of the particular environment which surrounds them. Functioning heart muscle cells of embryos when placed under the proper conditions in the tissue culture may be changed readily into simple cells which resemble the differentiated fibroblasts. Again under different conditions they may be made to grow and divide and resemble sarcoma cells and again by changing the mechanical conditions about them they may be changed back to rhythmically contracting cells, and vice versa.

From these observations it became evident that the general changes that the cells undergo in the normal organism from birth to maturity and death are not changes resulting from a gradual ageing of the cell, but changes induced by a gradual changing environment or by some more general formative force or forces operating in the organism. This idea is not a new one. It is found in the works of many of the earlier and later students of botany and general biology. Hofmeister, Sachs and DeBary had already appreciated this fact and it was the appreciation of it that led DeBary to his famous aphorism "Die Pflanze bildet Zellen, nicht die Zelle bildet Pflanzen." Whitman, Hertwig and many others had also come to the same conclusion. Driesch (1908) had noted that the growth of cells in the gastrula of the sea urchin is not independent but related always to a given form of the embryo. When he bisected the gastrulae of sea urchins he found that the halves do not grow at once, but only after they have each reformed by a shifting of cells and material into perfect gastrulae of one-half the normal size. The careful studies of Ribbert and later authors on cell division have shown that growth and cell division are not lost to body cells at the maturity of the animal, but recur at any time with the

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proper changes in the environment about the cells. Driesch and many others had also shown that if one separates the blastulae at the early divisions of the egg that each blastula will develop into a perfect animal; while if left undisturbed they develop only into one or the other of the special tissues of the single animal.

As it is evident, therefore, it is not the cell alone which is concerned in development. Growth, differentiation and function are also controlled by other more general conditions. The observations noted above indicate, as Wilson (1906) states, that the earlier conception of an independent body cell postulated by Schwann does not hold. The reaction of the cells in the body during normal development are under the control of other more general formative forces or stimuli. The question to be solved is the nature of these formative forces or stimuli. The solution lies in determining the nature of the environmental conditions suitable for growth, differentiation and function in body cells and a further knowledge of the immediate mechanisms of these processes as they exist in the cell.

Driesch, appreciating the existence of a general formative or molding force in the normal development of the animal, sought for the nature of this force. He thought that growth, differentiation and function are purely physico-chemical processes, which would be solved eventually. Failing to find evidence for the nature of the more general force which guides these processes in the body he thought it to be something apart from other known forces in nature. He named it "entelechy" and thought it to be a vital force not resolvable into terms of simple physico-chemical processes. He thus developed his neo-vitalistic theory of life.

Many other authors have not accepted this view. They have believed that this force will be resolved eventually into much simpler terms.

In a previous paper (Burrows, 1913) it has been shown that body cells can grow and divide in the tissue culture where blood plasma or a simple synthetic media free from any special stimulating substance is used, only when they are crowded into a small stagnant drop of any one of these media. Under these conditions this growth does not commence at once, but always after a given latent period. It can be stopped by separating the cells, by increasing the relative amount of medium about them or by washing the cells with a stream of serum or salt solution without disturbing the oxygen supply to them (Burrows, 1923a).

From these observations it became evident that an active independent growth of cells in the normal medium of the body depends on three factors: stagnation of medium, cell crowding and oxygen. When one washes the cells or places only a few cells in the stagnant medium these cells show migration. They decrease in size, engorge themselves with protein particles and fat droplets and stain more deeply. They do not grow and divide, but pass through the general changes peculiar to differentiation as it is seen in the body.

Function as seen through a study of muscle cells in the organism depends on still different conditions (Burrows, 1917–18; Burrows and Johnston, 1925b.) Embryonic heart muscle, striated muscle and smooth muscle cells in most cultures, migrate from the fragments into the medium as simple mesenchyme-like cells. From densely cellular fragments these cells grow and invade medium and have all the appearances and activities of sarcoma cells. From the less densely cellular fragments they differentiate into cells much like the fibroblasts of the adult animal. Rhythmical contractions develop in these cells only when they become stretched between the fragment and the clot or between a surface of the medium saturated with products of the fragment and an unattached part of the clot without. In other words, function obtains in these cells only when one end suffers the effect of stagnation and cell crowding and the other end is in a part of the medium largely free from such conditions and into which any products of the metabolism of the cell may diffuse away from it.

Differentiation as we have found it, therefore, is something peculiar to conditions of less crowding. It takes place more quickly when the cells are washed with serum. Function on the other hand is the result of a polarizing of the cell, a localization of stagnant conditions at one end of the cell and the freeing of the other end of these conditions.

In studying other types of muscle cells and other functioning tissues of the organism it has become evident that not only the rhythmical contractions of heart muscle are dependent upon this polarization of the cell, but that all types of function may be also dependent on the same conditions. The kind of function is peculiar to the type of cell polarized while the actual functioning is only the result of polarization. Smooth muscle and striated muscle cells behave as heart muscle cells in these cultures. They show rhythmical contractions only when one end is bathed by fluids from stagnant cell masses and the other end is free of these fluids. Under these conditions their contractions are not quick and rapid as heart and striated muscle, but slow and labored as those of smooth muscle in the body. In the body the functioning nerve fibres are stretched between the dense brain tissue and an end organ. Gland cells are stretched between a stagnant duct and a rich vascular supply without.

In studying these conditions of oxygen stagnation and cell crowding more carefully it was noticed that not only growth, differentiation and function are the direct result of various degrees and localization of stagnant conditions about the cells, but that all activity in the cell is dependent on these same conditions. When cells are overcrowded in a medium they show not only growth but a later self-digestion and destruction. When scattered in a large amount of medium they fail to show any changes whatsoever. They lie inactive but fully intact for an indefinite period under these latter conditions.

In analysing for the chemical significance of these facts it was found that an ample oxygen supply, crowding and stagnation, are important for the life of these cells because all of these activities are dependent upon the presence of certain concentrations of a substance or substances formed through the oxidation of certain substances by them. This substance or substances have been called the *archusia* (S). It is soluble in salt solution, blood plasma and the circulating and intercellular fluids of the body. The cell has no means to retain this substance. Its concentration at any time in the cultures as in the body is proportional to the number of cells forming it and the amount of medium diluting it. This substance can be readily extracted from any actively growing tissue of the culture or the body and its properties tested by adding such extracts to drops of plasma in a culture containing isolated and inactive cells.

These latter studies have shown that this substance in certain low concentrations, which have been designated as (S^1), has no effect. In medium concentrations (S^2) it causes the cells to migrate into a plasma medium, to engorge themselves with protein particles and small fat droplets and to show the differential changes noted above. Under the influence of this concentration of the archusia the connective tissue cells build intercellular substances and when such a concentration is localized at one end of a muscle cell it contracts rhythmically or functions.

In higher concentrations (S^3) growth and division of the cells obtain. In still higher concentrations (S^4) the cells suffer a self-digestion and destruction.

Applying these facts to the animal organism it became evident that the very active growth of the early embryonic life must be associated in part at least with the stagnant conditions of the embryo of this period. The slowing of this growth, the appearance of differentiation, the laying down of intercellular substances by the connective tissue cells and function are the result of the development of the blood vascular system. The guiding forces in the development of the body are those forces which regulate the development of blood vessels and the flow of blood through them. Cancer as clearly pointed out in other articles (Burrows, 1923a; 1924; 1925a) may be only the result of a relative reduction in the blood supply and a secondary crowding of the cells. It is only the result of the establishment of conditions about the cells, which allow them to form and maintain their archusia in an S^3 concentration.

The solution of the formative forces in development is the solution of the conditions which lead to the development of the blood vascular system and the proper regulation of the concentration and localization of the archusia (S). Body cells are not different from other unicellular organisms in nature as far as their fundamental reactions are concerned. It is in the stagnant pool and not in the running stream that unicellular life abounds.

Normal development is something imposed on the cells of the organism by the blood vascular system. Remove this blood flow without destroying the oxygen supply to the cells and they return to their primitive state. This is seen by placing fragments of tissue in small stagnant drops of medium in a tissue culture. Cancer is merely this primitive state.

In reviewing this particular system, therefore, as it had built itself before us through the analysis just described, we had wondered at first whether Driesch may not have been correct in assuming that the molding or formative force of the organism is something quite apart from those forces active in the cell. The pictures of differentiation and function as we have casually observed them above, indicate that they are changes which take place at the expense of the growth reaction of the cell, at the expense of the whole life of the cell itself. It was difficult, therefore, to see how any other than a wholly different outside force could impose such conditions on the cell.

It was not until one of us had studied more carefully the exact nature of the growth reaction of the cell and the amount of cell crowding and stagnation necessary for growth in the organism that we appreciated that this may not be altogether true. Cellular growth as it occurs in the normal organism is not an independent growth of cells but a carefully regulated one. In the previous studies as outlined above it had been found that body cells grow not only when they are crowded into narrow stagnant confines in which case they can form and retain their own archusia, but these same cells can be made to grow when isolated in a large bulk of medium by adding archusia from other sources to the medium. In the first case the growth is independent as in cancer. In the second place it is dependent on the source of the archusia and must stop with a stopping of the supply unless the cells proliferating have formed into a mass large enough to maintain their own archusia in sufficient quantities for the continuation of their growth. In the body such is impossible because the blood vessels keep pace with the general growth of the tissues and prevent the stagnation necessary for such an independent growth.

The growth of the blood vascular system is only the growth of the endothelial cells. This growth of the endothelial cells is not independent, but also carefully regulated as the growth of the other tissues. The cells in cancerous tissues and in the earliest periods of development are alone crowded sufficiently and their environment sufficiently stagnant for them to grow independently. In studying the reaction of the blastomeres of chick and frog-embryos in cultures, it was interesting to note, however, that these cells are unable to react when simply crowded into a stagnant medium as the cells of the later developmental periods and adult life. Masses of blastomeres of these embryos placed in a culture medium have failed to grow where similar masses of cells of older embryos and cancer

have grown readily and independently of any stimulating substance. These blastomeres evidently contain an inhibitor or inhibitors which prevent their forming ample archusia for growth. While we do not know the nature of this growth inhibiting substance or substances in these blastomeres, it disappears from these cells with the disappearance of the yolk granule. These yolk granules disappear from the cells of chick-embryos at an early period. Immediately after their disappearance the cells react by growing when transplanted in sufficient numbers to stagnant drops of plasma. In the frog these granules disappear more slowly. The chick's cells of a much earlier period than the frog's cells grow when transplanted in sufficient numbers to the culture medium (Burrows, unpublished notes).

With the disappearance of the yolk granules in the normal developing embryo the cells are already too well separated by the blood vessels to allow the stagnation necessary for an independent growth. There is no time, therefore, in normal development when the stagnation is sufficient for the cells to grow independently.

If our measurements are correct body growth cannot take place, therefore, at any period without outside aid in the form of a definite growth stimulus. The question that confronted us then was the source and nature of this stimulus.

In analyses made by one of us (Burrows, unpublished notes) it has been found that the archusia (S) from connective tissue cells will not only stimulate connective tissue, but other cells of the organism. It was further noted that body cells can be stimulated to grow by the archusia extracted from widely different species of animals and from cultures of growing bacteria.

In an analysis of the development of cancer one of us (Burrows, 1923; 1925a) has found that cancer may be developed in the organism by localizing extracts containing the archusia. When extracts of the archusia are injected into the skin of normal animals they escape readily by the way of the blood stream and have no effect. When the circulation is first impeded it remains and stimulates an active growth of the cells. The cells stimulated are the cells between the blood vessels. These growing cells form no blood vessels, but grow to form a dense mass. When such a mass is large enough and sufficiently stagnant it then continues its growth independently. When less stimulus is added the connective tissue cells (fibroblasts) do not grow, but lay down an excess of intercellular substances, fibrils. These tumors do not become malignant, but degenerate eventually into hyaline scars (Burrows and Johnston, 1925b). In the adult skin the epithelial layer is already stagnant and cellular. Its cells can be stimulated to grow with less archusia added from without. Cancer develops, therefore, more readily in this tissue than in the connective tissues of later life.

Blumenthal, Auler and Meyer (1924) have shown that similar malignant tumors can be developed by cultures of the B. tumefaciens and other bacteria. Most bacteria liberate not only a growth stimulus, but other toxic products. These latter substances are liberated through secondary fermentation. The formation of alcohol by yeast is an example. The B. tumefaciens liberates no such secondary products. They injected this organism together with kieselguhr and the fluid from a cancer, which is rich in archusia (S). Tumors developed. These tumors were removed, cut into pieces and transplanted into the tissues of other animals. Eventually they became malignant tumors. In the experiments performed by one of us the circulation was reduced by first injecting fragments of skin and intestines of older embryo rats into the skin of adult rats (Burrows, 1923; 1925a). These fragments grew to form benign cystic tumors. They cause a reduction in the circulation, atrophy and ulceration of the skin. Either a Berkefeld filtrate of a Jensen sarcoma or an extract of young, actively growing rat embryos was introduced into this area. In each case the epidermis of the skin suffering the reduced blood supply suffered an active hyperplasia. In most cases this eventually receded. In two cases where the filtrate of the sarcoma was used typical carcinomata developed. In none of these cases were the blood vessels stimulated to grow. The cells between the blood vessels alone suffer stimulation. In the blood vessels the stimulus always escapes by way of the blood stream and cannot remain in a concentration sufficient for growth.

In the light of these studies on cancer it became evident that the functioning tissues of the body may owe their origin to the same conditions. As noted above differentiation and function are the direct result of the development of the blood vascular system. From the work on cancer it has been shown that the introduction of a stimulus between the blood vessels produces the development of a non-vascular cellular mass, or cancer. This same stimulus introduced into the blood vessels should stimulate them to develop more rapidly than the cells without. This must cause them to overgrow the other cells and give rise to a vascular and functioning tissue. As stated above, the egg cell contains inhibitors which prevent the blastomeres from growing when crowded in the same proportion consistent for a growth of body cells at a later period. After the initial stimulation of fertilization the embryo dies unless it becomes connected with the yolk or the mother. Channels develop in it which later carry fluids from the yolk or the mother's uterus. These are the blood vascular system. In the plant these channels extend from the roots to the leaves. They are not the result of overcrowding and an excess of the archusia in the embryo, but their development is associated with a decrease in this stimulating substance. The blastomeres do not form this substance as readily

as the cells of a later period. These channels may be, therefore, the result of the archusia acting from without on these systems (Burrows, 1925b; and Jorstad, 1925).

It is now well established that the body cannot exist alone on proteins, fats, carbohydrates, salts, water and oxygen. Other accessory food factors are also necessary. These accessory food factors have been called vitamins. The chief of these are vitamins A and B. C is essential for the life of man, but not for rats. D and E are probably concerned with the differentiation of special tissues as iodine is essential for the development and function of the thyroid gland. The nature of the action of vitamins A and B and their source has not been determined. It has been shown, however, that vitamin B stimulates the growth of bacteria while vitamin A retards it (Manville, 1925). The question arose, may vitamin B be only the archusia (S) of other cells in nature or the stimulus which has been found by the above studies necessary for growth and maintenance of life in higher animals. It has been shown that vitamin B is water soluble. The archusia has also a similar solubility. To give more absolute proof of this deduction it became of interest, therefore, to study the effect of feeding as vitamin substitutes, bacteria such as the B. tumefaciens (Pseudomonas tumefaciens) known to stimulate cells when injected into the body, other bacteria and tissues of animals known to be rich in archusia.

In making these tests we appreciated that these tissues contain many other substances than the *archusia*. Since it has been impossible at the present time, to isolate the *archusia* in pure form as it has been impossible to isolate the vitamins, it was necessary in order to give evidence that the *archusia* is the same as one or the other of the vitamins, to use tissues containing different quantities of the *archusia* and to show that as the *archusia* value varies the vitamin value also varies.

The general action of the *archusia* in the cells has been outlined above. Malignant tumor tissue contains very large quantities of it. A similar high content is found in the tissue of 5-day-old chick-embryo. The growth of the embryo is most rapid at this age. This growth rate decreases as the embryos age. Ten day old chick-embryos contain less *archusia*. There is considerably less in embryos 15 days old. With this decrease in the *archusia* value in the ageing embryo it has been interesting to note, however, that the extracts of these embryos become more efficient in preserving the life of other cells in vitro. Extracts of malignant cancer cause the cells to grow rapidly and then disintegrate within a few hours. A very similar rapid growth and disintegration is seen when extracts of 5-day-old chick-embryo are added to the medium of a culture. Extracts of older embryos stimulate the cells to grow much less actively. These growing cells stain much more sharply than those placed in the extracts of the younger embryos. By transplanting such cells at regular intervals

into a fresh medium containing these extracts they can be kept growing for an indefinite time. In the cultures where extracts of malignant tumors and the younger embryos are used the cells are always sooner or later destroyed. If this fails in the first culture they are destroyed in the second. The destruction of the cells is that of a digestion from an over abundance of the *archusia*.

Noting these differences in the *archusia* and the growth promoting value of tissues it became of interest in testing these tissues for their vitamin values to study not only cultures of B. tumefaciens and other growing organisms, cancerous and embryonic tissues, but to compare carefully the value of embryos of various ages to see if their vitamin value varies as their stimulating value varies in the cultures.

Experimental data. The rats used for these experiments were young healthy animals from our inbred stock. Previous authors had already shown that rats grow readily on the following diet:

	grams
Potato starch	80
Egg albumin scales	50
Salts (McCollum)	10
Butter	30
Vegex	10

Vitamin C is not essential for the growth of rats.

This diet contains no vitamin A except as it is present in the butter. When Crisco, a commercial vegetable fat product is substituted for the butter the diet becomes deficient in vitamin A. The source of vitamin B in this diet is the vegex, a commercial autolysed yeast product furnished us by the Vitamin Food Co. of America, Westfield, Massachusetts.

Experiment 1. In this experiment one 15-day-old chick-embryo, carefully ground in a mortar, was substituted for the vegex in each of the following diets:

	grams
A. Potato starch	80
Egg albumin scales	50
Salts (McCollum)	10
Butter	30
15-day chick-embryo	1
B. Potato starch	80
Egg albumin scales	50
Salts (McCollum)	10
Crisco	30
15-day-old chick-embryo	1

Diet A is deficient in vitamin B except as it is present in the embryo. Diet B is deficient in both vitamins A and B except as they are present in the embryo. Each of these diets was one week's ration for two rats. The

results of the action of the diet were determined by the general appearance of the rats, the development of xerophthalmia, etc., and the weight. Two sets of these experiments were performed, one in the fall of 1924 and the other in the spring of 1925. The results of the experiment performed in the fall of 1924 are given in figure 1. The rats fed on Crisco show marked loss of vigor. Their hair became roughened, but they did not develop xerophthalmia even at the end of the experiment. The growth curve in both of the experiments was practically the same. These embryos contain much less vitamin B than those of the earlier period and more vitamin A.

Experiment 2. In this experiment five 10-day-old chick-embryos were substituted for the one 15-day-old embryo in the same diets and fed at the same time to closely related rats of about the same size. The growth of the rats in one of these experiments is shown in figure 2. These embryos contained more B than the 15-day embryo, but relatively less A. The animals fed on Crisco developed eye signs on the 73rd day, and showed roughened coats much earlier.

Experiment 3. In this experiment thirty 5-day-old chick-embryos were fed instead of the one 15-day-old embryo to rats on the same diet otherwise. The results of one of these experiments are shown in figure 3. These embryos contain still larger quantities of B and less A than the 10 or 15-day-old embryos. Xerophthalmia developed in these rats on the 70th day.

Experiment 4. In this experiment 10 grams of a Jensen sarcoma were added in place of the chick-embryo in the diets given above. The sarcoma used was obtained from the Cancer Research Laboratories of Columbia University. It was transplanted to our stock a year ago and has continued to take and grow actively in about 50 per cent of the transplants. In making these experiments the entire tumor tissue was used. These tumors suffer considerable central necrosis and liquefaction. This central necrotic area was ground in the food with the peripheral lining and actively growing parts. The results of one of these experiments are given in figure 4. It is interesting that this tissue differs from the embryo in that it contains no A. It acts the same as the cultures of actively growing bacteria.

Experiment 5. In a previous article (Burrows, 1925b) it had been pointed out that 20 cc. of a 2-day-old culture of B. tumefaciens contain ample quantity of vitamin B for a normal growth of rats. The cultures were prepared in a natural potato decoction which was autoclaved twice. This medium contains vitamin B but not in such quantities as when the bacteria are present.

In these experiments we have repeated our earlier ones using slightly older cultures. The results have been the same as those shown in the previous experiment and are practically identical to those of the sarcoma. These cultures supply B but no A, figure 5.

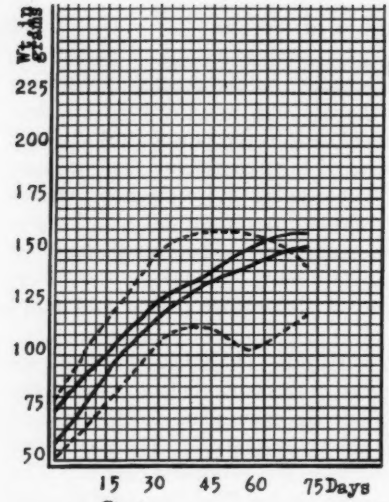

Fig. 1

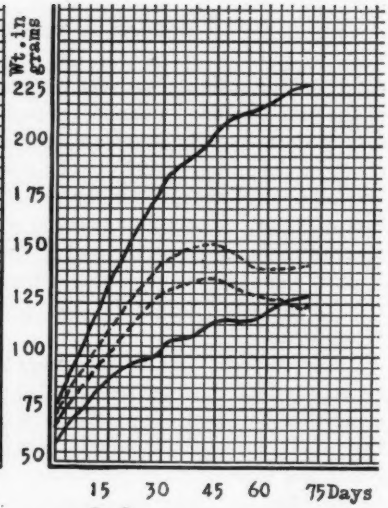

Fig. 2

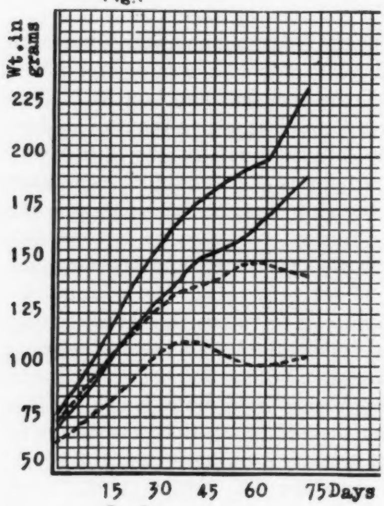

Fig. 3

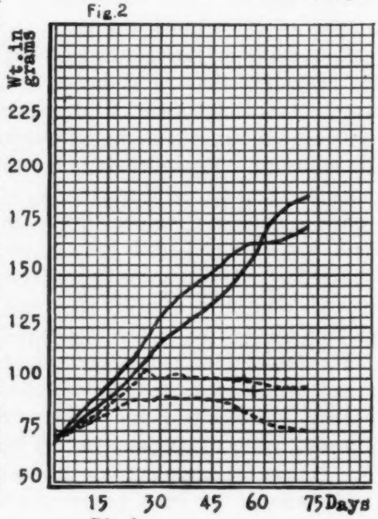

Fig. 4

Fig. 1. The effect on the growth of male rats of feeding a 15-day-old chick-embryo as vitamin substitute. Experiment performed in fall and winter of 1924. ------ Crisco in diet. ——— butter in diet.

Fig. 2. The effect on the growth of male rats of feeding 10-day-old chick-embryos as vitamin substitute. Experiment performed in spring of 1925. ------ Crisco in diet. ——— butter in diet.

Fig. 3. The effect on the growth of male rats of feeding 5 day old chick-embryos as vitamin substitute. Experiment performed in fall and winter of 1924. ------ Crisco in diet. ——— butter in diet.

Fig. 4. The effect on the growth of female rats of feeding Jensen sarcoma of rats. The experiments with sarcoma were performed in the spring of 1925. ------ Crisco in diet. ——— butter in diet.

Experiment 6. To make certain that the similarity of the curve of this cancer producing organism and the cancerous tissue are not related, but merely the curve produced by any actively growing cells, similar experiments were performed with the B. campestris. This bacteria produces the black rot of plants. Chambers (1925) studied the properties of this organism in the laboratory here and found that its pathogenic properties are related to its liberating a starch splitting ferment. The action of this ferment does not appear at once in the cultures, but only after 5 days or longer.

In a previous paper (Burrows, 1925b) we have shown that 2 day old cultures of this organism provide vitamin B in the same manner as the B. tumefaciens. We have repeated these experiments to find similar curves when 5 to 7 day old cultures are fed.

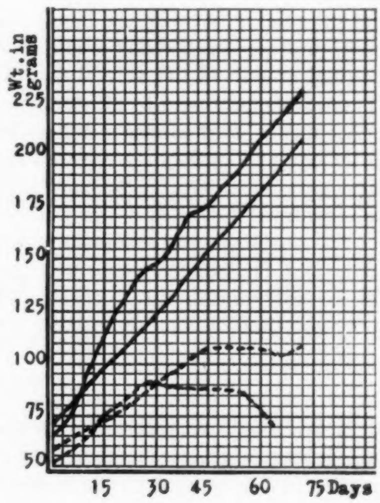

Fig. 5. The effect on the growth of male rats of feeding 5 to 7 day old cultures of B. tumefaciens as vitamin substitute. The experiment was performed in the fall of 1924. ------ Crisco in diet. ——— butter in diet.

Summary and discussion. The above studies have shown that the tissues which stimulate cells to grow most vigorously in the tissue culture act as pure vitamin B substitutes when fed to animals. The extracts of tissue of the sarcoma are most rich in the stimulating substance, the archusia. These tissues when fed are shown to contain little or no vitamin A but act as pure vitamin B substitutes. The next most actively growing cells are found in fragment of 5-day chick-embryos. As the embryo ages this stimulating value for the cells of the culture wanes. It is interesting to note that this decrease in stimulating value is to be related not only to a decrease in vitamin B but an increase in vitamin A.

This high vitamin B value without any noticeable vitamin A is seen not only in these actively growing tissues of the body, but also in the culture of the actively growing tumor forming organism, B. tumefaciens, and in the actively growing cultures of the non-tumor forming organism, B. campestris. While it is impossible to prove without working with pure products that the archusia (S) of the cell is vitamin B there is no evidence from these experiments that it is otherwise. Our test for the *archusia* is the ability

of a cellular exudate to stimulate activity in tissue cells. The highest stimulating extracts are obtained from the most actively growing cells, cancer, young embryos and actively growing young cultures of the bacteria. These have been found to have the greatest amount of vitamin B. Hand in hand with the decrease in the *archusia* and the growth rate of the ageing embryonic tissues the vitamin B value decreases. With this decrease in the vitamin B, the vitamin A value increases.

While we do not know the exact significance of these studies at the present time it is certain that any one of the above substances when injected into the tissue between the blood vessels stimulates the cells to grow and may produce a cancerous organization. The same when fed stimulate a normal growth of the animal and act as a perfect vitamin B substitute. These facts only tend to substantiate, therefore, our former deductions by other means, that cancer is none other than the normal growth that body cells enjoy when they are released from the effects of an active circulation and crowded into a sufficiently stagnant environment. By these same observations it seems evident that one of the chief of the formative forces of the organism, the existence of which was so fully appreciated by the botanists, Hofmeister, Sachs and DeBary and by many of the zoölogists at this early and in later periods, Whitman, Hertwig, Driesch and others may also find explanation in these same terms.

CONCLUSIONS

1. Body cells can grow only when acted upon by a certain concentration of a substance or substances formed by them or by other cells. This substance has been called the archusia (S).

2. The archusia (S) is necessary not only for growth but all activities of the cell. In concentrations lower than that necessary for growth it induces differentiation and function.

3. In the normal developing organism it does not exist in quantities sufficient for the early growth and later differentiation and function. This growth is dependent on a supply of it from other sources.

4. This substance formed by body cells is not specific. Body cells can be made to grow by the archusia formed by bacteria and other plant and animal cells.

5. Extracts of tissues and bacteria containing various quantities of archusia (S) as tested on tissue cells act in the same proportions as vitamin B substitute in the diet of animals. Vitamin B is probably only the normal energy for life (the archusia (S)) formed by all cells in nature. It is the stimulus necessary for the normal growth, differentiation and function of the body.

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ON THE SOURCE OF VITAMIN A IN NATURE[1]

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It has now been shown that while vitamin A is essential for the growth, proper structure and function of higher metazoan life, it is not needed by yeast, bacteria and many other lower unicellular forms. This substance is present in green leaves and seeds, but not common in other parts of plants. Recently Cramer (1924) has found that vitamins are also not essential for the growth of cancer cells. In repeating these experiments we have found that when one or the other of the vitamins is removed from the food of an animal having cancer, the cancer grows on undisturbed while the animal, on the other hand, suffers materially from this reduction (Burrows and Jorstad, unpublished notes).

In conjunction with these particular facts it is also interesting to note that the protoplasm of bacteria and other unicellular organisms is immortal while the cells, excepting the sex cells, of higher animals have a very definite span of life. Death to these cells is as certain as their growth and differentiation unless they become cancerous. As L. Loeb (1901), (1915) has pointed out, the cancer cell probably has an immortality the same as the cells of lower animals. At least many animal cancers have been transplanted from animal to animal for a very long time. These cells have continued to grow actively after each transplant and over a period of many generations of the animal from which they arose.

It became evident from these observations that vitamins are something necessary for the differentiated cells of higher animals, but not for the growth of these cells as they are seen in cancer, or for the simple growth and reproduction of unicellular organisms in nature. To understand the significance of vitamins and their source in nature we must first understand, therefore, the nature of the mechanisms of growth, differentiation and function in tissue cells.

Previous experience and experiments have shown that bacteria and many of the unicellular organisms which can grow independently of any

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specific aid in the form of vitamins in nature grow only in stagnant pools. They are destroyed in running streams. Wildiers (1902) had noted that a few yeast cells will not grow when added to a wine cast. Yeast grows in these casts only when a considerable number of cells are added. He analyzed for the significance of these facts to find that the growth of yeast depends on a certain amount of a substance *bios* in the cast. This *bios* is formed by the yeast cells. It may be extracted from them. When extracted from other yeast and added with a few yeast cells to the cast the few cells will then grow. Otherwise a large number of yeast cells must be added or they will succumb before they can bring the *bios* to a concentration proper for their growth.

These same facts applied to bacteriology have been used practically for the destruction of sewage. It has been found that bacteria are readily destroyed in running streams.

In 1913 without appreciating the significance of the earlier work on sewage destruction and the work of Wildiers, one of us (M. T. B.) had noted that body cells when isolated from the body in a tissue culture cannot grow without the addition of extracts of an actively growing tissue unless they are crowded into a small stagnant mass of medium (plasma) supplied with oxygen. The growth can be stopped by decreasing the number of cells per unit area in the mass, increasing the amount of absorbing medium or by washing the cells with a stream of serum or salt solution.

At first it was thought that these factors of stagnation and cell crowding are important because the plasma contains inhibiting substances. It has been found that this is not true. These factors of stagnation and cell crowding are as important for growth in simple synthetic media and salt solution as in plasma.

When the cells are so crowded together the growth does not commence at once, but always after a given latent period. This latent period is longest about fragments of tissue which were not previously growing in the body before their transplantation to the culture and shortest about fragments of already actively growing cells. This growth is dependent upon the accumulation of substances or a substance in these stagnant crowded cultures. This substance or substances has been called the archusia (S) or the driving substance of the cell. It is formed by the cells of the body only in the presence of oxygen. (Burrows, 1921, 1923, 1924, 1925a, to appear. Burrows and Johnston, 1925.)

This substance or substances, the archusia (S) is essential not only for the growth of cells, but for all activity in these cellular elements. It is soluble in the circulating blood of the body. The cells can form it, but they have no means to retain or localize it. It can be readily extracted with salt solution from an actively growing tissue. Its effect upon the

cells can thus be readily ascertained by observing the effects of various concentrations of such extracts on isolated cells in a tissue culture.

Such studies have shown that in low concentrations S^1 the archusia has no effect. In medium concentrations S^2 it reacts with the protoplasm of the cell to cause it to liberate a soap-like substance, the ergusia. The ergusia is split off from the protoplasm of the cell itself. The ergusia is readily absorbed by proteins and fats which are not already saturated with it. It decreases the surface tension of the cell in the presence of these substances. Cells liberating the ergusia under an S^2 concentration of the archusia move away from other cells when placed in a plasma clot which absorbs the ergusia. They also move towards larger droplets of fat placed in this medium and draw smaller more mobile droplets of fat and protein particles into their protoplasm.

The ergusia is an active blood coagulant. It transforms the fibrinogen of the blood into fibrin. The epithelial cells liberate not only ergusia but also an active proteolytic ferment which dissolves the fibrin as it is formed. The connective tissue cells liberate no such ferments. The fibrin formed by them in the exudate remains under these low concentrations (S^2) of the archusia and become transformed into the extra-cellular fibrils peculiar to the connective tissue cells throughout the body, the matrix of bone, the extracellular fibrils of connective tissue, etc. (Hertzler, 1919, Baitsell, 1916, 1917, and Burrows, 1916–17.)

Function maintains in body cells when the archusia is not equally distributed about the cells, but localized in an S^2 or slightly higher concentration at one end of the cell and absent from the other sides and end. It occurs when the cell is placed in an environment where the archusia can escape from all sides of the cells except over one small area. This area suffers a liberation of the ergusia, a decrease in surface tension and a change in its electrical state. Such local areas of change in surface tension and electric state are readjusted by explosive break downs. These explosive break downs are manifested as function (Burrows and Johnston, 1925).

Under the influence of an S^2 concentration of the archusia the cell liberates ergusia, but does not form it. Heart muscle cells contracting rhythmically in the cultures suffer a continuous loss of their ergusia and exhaustion in a few hours or days. These same cells and other cells liberating ergusia and migrating shrink in size and soon become exhausted in the cultures. Only under the influence of a higher concentration (S^3) of the archusia are the cells able to digest proteins and fats and synthesize ergusia and the protein elements of their protoplasm, grow and divide.

In the exhaustion of the cell in an S^2 concentration of the archusia it is interesting to note, however, that the proteins are apparently undisturbed. As the cell shrinks it continues to stain more and more sharply

with stains absorbed by proteins. Only the soap, the ergusia, is lost to these cells.

Analyzing the growth of the body in terms of these observations it has already been pointed out that a concentration of archusia as high as S^3 exists generally in the body only in the earliest period of development and in cancer. (Burrows and Jorstad, 1926, this issue.) In the normal organism this high concentration of the archusia decreases progressively with the development of the blood vascular system. This decrease is noted first in the connective tissue and muscle areas. The cells in these areas cease to grow in the early periods of embryonic life. The subsequent growth of this tissue is merely the enlargement of the fibers in the case of muscle and the laying down of intercellular substances in the case of bone, tendons, reticular and other of the connective tissues. These connective tissue fibrils as clearly pointed out above and which is probably also true for the greater part of the muscle fibers are merely extracellular proteins, fibrinogen, coagulated by the ergusia liberated by the connective tissue cells.

In the more densely cellular and stagnant epithelial layers the archusia does not decline so early. Only at maturity does it decrease below the level consistent for growth excepting in certain regions; such as skin epithelium, the bone marrow, the nails, hair and sex glands. Growth maintains in these regions as already pointed out elsewhere, because they have a much poorer circulation than the remaining parts of the body (Burrows, 1923).

As stated in the accompanying paper on the "Source of Vitamin B in Nature", this high content of the archusia (S) in the normal organism is not maintained alone, however, at any time by the cells themselves. While the blastomeres are sufficiently crowded for other cells to form and maintain a concentration of S^3 these cells cannot form this substance so rapidly. In all later periods excepting in cancer the circulation is too great and cells too much separated to maintain such conditions. The immediate concentration of the archusia in these tissues is sustained by an added supply from the outside. The archusia (S) thus supplied is that formed by other plants and animals and occurs in the food and is recognized as vitamin B. In cancer alone is the concentration of cells and the stagnation of circulation adequate for the cells themselves to maintain the archusia in a concentration sufficient for growth.

Not only from the fact that the connective tissue cells do not grow and multiply after the earliest periods of development, but also from the careful study of extracts of these tissues from young and older embryos and adults well supplied with vitamin B it has become certain that these tissues do not contain archusia above an S^2 concentration even under the most favorable conditions. Only in the case of injury and a destruction

of the blood vessels does the archusia reach an S^3 concentration and an actual growth of cells obtain in these tissues. The same is true of many of the functioning epithelial tissues of the adult body.

As noted above, the connective tissue and muscle cells in the tissue culture under these conditions of an S^2 concentration become exhausted of their ergusia in a few hours or a very few days. In the body this is not true. They continue to lay down intercellular substances and become more and more separated for a period of several years. The question arose, how is this possible. Do they obtain a supply of this soap-like substance, the ergusia, from other growing cellular systems as they obtain the archusia (S)?

The ergusia is a fat soluble substance split from their protoplasm. It has in this regard the general properties of vitamin A. If the archusia is vitamin B the question arose, may not vitamin A be only a supply of the ergusia formed by growing cells in nature.

To prove this possibility it became of interest in the first series of experiments to remove the ergusia from tissue cells and see if it can be replaced by vitamin A. In the second series of experiments we have attempted to ascertain more directly that vitamin A is formed in the growth reactions of cells and is obtained from other growing cells in nature. The work of recent authors might lead us to believe that it is formed from fats by ultra violet rays. A more careful analysis of the growth of tissue cells as cited above and the actual facts known concerning vitamins indicate, however, that this may not be true.

The effect of vitamin A on cells from which the ergusia is being removed. One of us, (Jorstad, 1925a) then undertook the first problem. It had been noticed by the senior author that when cells are placed in contact with fibrinogen or drops of fat which absorb their ergusia they move towards the absorbant and shrink in size and may disintegrate as they lose their ergusia to the absorbant (Burrows, to appear). Jorstad found that drops of coal tar placed in the tissue also drag the neighboring cells of the tissue to them and lead to a shrinking and degeneration of these cells exactly as other absorbants of the ergusia had acted. Other fats we find also act in the same capacity (Burrows and Johnston, 1925). In the cultures it was noticed that fibrinogen and animal fat can affect the cells only until they are saturated with the ergusia or a certain distribution factor is satisfied between them and the cells. Jorstad finds the action of coal tar is also limited in time. He further notes that the amount of deterioration suffered by each cell is less when the drop of tar is placed in a cellular tissue than when it is placed in a less cellular one.

In the light of the above deduction (1925b) he then studied the action of tar on the cells of animals fed on a diet deficient in vitamin A and in similar animals fed on a diet rich in vitamin A. In the animals fed on a

diet deficient in vitamin A the cells degenerated completely as they moved towards the tar. In the animal fed a diet rich in vitamin A they remained intact and as they became crowded about the tar and as their archusia increased in amount about them they grew actively and divided by mitoses. These observations showed definitely, therefore, that the ergusia lost to cells can be replaced by vitamin A.

Production of vitamin A by bacteria. As noted above, previous authors have found that bacteria, yeast and other unicellular forms may grow readily in a medium free from vitamin A. Body cells when crowded together are also able to grow in a medium free from this substance. In spite of this fact it is interesting to note in the accompanying article (On the Source of Vitamin B in Nature) that cultures of actively growing bacteria supply vitamin B but no vitamin A when fed to animals. The question arose was this due to an absence of vitamin A in these cells or to the fact that it was combined in such a way that it could not be used by the animal.

At an earlier time it has been noticed by one of us (Burrows, 1916–17) that the border cells of a fragment of a cellular tissue which had migrated away from the fragment into the medium of the culture do not grow except when they are caught in a film of a soap-like substance liberated by cells suffering digestion in the center of the fragment. This digestion of cells in the center of the fragment had been studied. It was found not to be an autolysis from the absence of oxygen. It takes place in the presence of oxygen. It is the result of an excess of the archusia. As noted above when the cells are placed in a medium containing an S^2 concentration of the archusia they liberate a soap-like substance the ergusia. The ergusia is not soluble in water nor can it decrease the surface tension of water. It is absorbed only by proteins, fats and certain other substances. In an S^3 concentration of the archusia the cells digest the proteins and fats stored in their protoplasm and about them. With this digestion they absorb water, grow and divide. In higher concentrations of the archusia (S^4) they disintegrate and liberate another soap-like substance. This soap differs from the ergusia in that it has strong affinities for water. It spreads rapidly over the surface of water. Otherwise it apparently has the same affinities for proteins and fats. The question arose, may not this soap-like substance be similar or the primary synthesis from which the ergusia may later arise.

In our previous studies we had fed always young actively growing cultures of bacteria. In these experiments it became of interest not only to repeat our earlier ones with young cultures, but also to feed older cultures of the organism or cultures in which the bacteria had overgrown the medium and were dying off in it. The bacteria used in the earlier experiments were pseudomonas tumefaciens and pseudomonas campestris. These

bacteria are also known as B. tumefaciens and B. campestris. We will continue to use the latter names so as to maintain a uniformity of terms in our articles.

The bacteria were grown in 500 cc. flasks containing 100 cc. of a simple potato decoction. The decoction is made by cutting up pealed potatoes into small pieces; 250 grams of the potato are placed in 1000 cc. of water. The mixture is autoclaved at 15 lbs. pressure for 10 minutes and allowed to stand. After 24 hours the liquid is filtered off, made up to 1000 cc., placed in flasks and again autoclaved at 15 lbs. pressure for 10 minutes. Both bacteria grow readily in this medium at a temperature of 28°C. The medium contains small quantities of vitamin B, but no vitamin A.

In the earlier experiments we substituted 20 or 40 cc. of a 2- and 5-day-old culture in the following diets:

1.	Potato starch	80 grams
	Egg albumin scales	50 grams
	Salts (McCollum)	10 grams
	Butter	30 grams
	Whole culture of one of the bacteria	20 or 40 cc.
2.	Potato starch	80 grams
	Egg albumin scales	50 grams
	Salts (McCollum)	10 grams
	Crisco	20 grams
	Whole culture of one of the bacteria	20 or 40 cc.

In these experiments we tested not only the effect of adding 40 cc. of these younger cultures to the diet, but the same amount of older and older cultures of these bacteria. Diet 1 contains ample quantities of vitamin A in the butter, but no vitamin B except as it is contained in the culture of the bacteria. Diet 2 contains neither vitamin A nor vitamin B except as they are contained in the culture of bacteria.

The quantity of materials given in the above diet is sufficient to feed two rats for from 5 days to one week. Young healthy rats of our stock which are closely related were used for these experiments.

As must be pointed out here vitamin A, according to the deductions from our experiments, is necessary only for the formation of the ergusia in the differentiated cell. The ergusia is a part of the protoplasm manifactured by the cell only in the presence of a high concentration (S^3) of the archusia. It is liberated by these same cells only in a higher concentration (S^4) of the archusia. The archusia is the energy for its formation and derived from the cell. As the cultures age and the food is used up the archusia content must not only decrease, but it must also decrease with the formation of the ergusia and according to the Law of Mass Action it must appear as the substances which it forms increase in quantity.

In the previous articles we have shown that 2 and 5 to 7 day old cultures of B. tumefaciens are rich in vitamin B and that the vitamin B content and archusia content run hand in hand. In these experiments it was found that when 9 day old cultures of B. tumefaciens are fed, the vitamin B value has not decreased or increased over that of the 2 day old cultures. The cultures contain no vitamin A.

When 18 day old cultures are fed the picture changes. The rats grew as well on the diet containing neither vitamin B nor vitamin A as on the

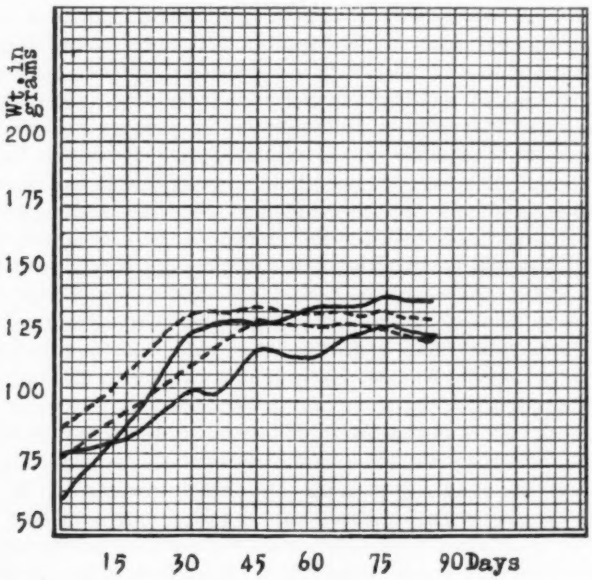

Fig. 1. Growth curves of animals fed on diets in which 18 day old cultures of B. tumefaciens were substituted for the source of vitamin B and vitamins B and A respectively. - - - - - Crisco in diet. ———— butter in diet.

diet containing vitamin A, figure 1. These rats maintained not only their weight, but they showed no evidence of age changes and their coats remained glossy. Their bones showed no evidence of rickets.

Chambers (1925) working in this laboratory had studied carefully the growth of this organism. He found that it reaches its maximum growth in cultures as we have described them soon after 5 to 9 days. Subsequently the cultures become filled with non-viable bacteria. We have found with these latter changes a rise in the vitamin A content of the cultures.

We also studied the effect of feeding cultures of the B. campestris in the same manner. Chambers had also studied the cultural characteristics of this organism while working here in the laboratory. The B. tumefaciens when injected into plants stimulates a growth of the cell and produces the crown gall. The campestris on the other hand produces the black rot of plants. Chambers accounted for the destruction of the plant tissue by the B. campestris through the fact that this organism splits the starch of the plant cells.

In previous papers (loc. cit.) we had shown that B. campestris produces vitamin B the same as the B. tumefaciens. Chambers finds that the starch splitting properties of this organism does not become pronounced in the cultures until after 5 to 7 days of growth. In feeding 18 day cultures of this organism it was interesting to note that the animals survived much longer on these older cultures than on the younger ones when vitamin A is left out of the diet. At 84 days these animals had developed no eye symptoms. They had gained little in weight and had eventually lost considerable weight, but they remained alert and their hair remained smooth. The B. campestris does not multiply so rapidly in the cultures as the B. tumefaciens. Whether this loss of weight is the result of toxic substances liberated in the secondary fermentations instituted by this organism or to the few bacteria growing in the culture has not been determined. There seems little doubt, however, that a certain amount of A is liberated by these bacteria as the medium is overgrown and the bacteria suffer selfdigestion.

Discussion. In recent years it has been fully shown that certain food substances other than proteins, fats, carbohydrates, salts and water are necessary for the proper growth and life of higher animals. Why these substances are necessary for these animals and from whence they are derived in nature has not been determined. These substances have been called vitamins or accessary food factors. According to the classification used by the American authors five such substances have now been identified. These have been designated as vitamin A, B, C, D and E (McCollum and Simmonds, 1925). The first two of these substances A and B are essential for the growth of all higher animals and plants. B aids in the growth of lower bacterial forms while A retards the growth of these organisms. C is necessary for the proper growth and development of man, guinea pigs and certain other animals, but not for the growth and development of the rat. D has been recently identified as essential for the growth of bone. Evans and Bishop (1923) claim a special vitamin E necessary for the function of the sexual organs.

It is evident from these facts that a variety of substances have been classed together under this one heading which may be found to be widely different in their physiological action as well as chemical constitution.

If D and E are proven to exist, they belong in a class quite different from A and B. They act for the differentiation of special tissues and not for the general life of the animal. They belong, therefore, in a class with iodine, which is essential for the differentiation of the thyroid gland. Where C belongs has not been determined.

It has been taught since the days of Claude Bernard that the cells of the body grow continuously and replace the loss suffered by them through the wear and tear of function. A careful study of the cells of the body in the tissue culture has shown that this is not the case. The production of protoplasm by the cells takes place only when the cells are bathed by a certain concentration of a substance which has been named the archusia (S). The archusia (S) is formed by all cells oxidizing food substances. The cells cannot retain this substance. It is soluble in salt solution, water and body fluids. Its concentration at all times depends on the number of cells forming it and the absence of means for its escape. When a sufficient number of cells are placed in a small stagnant drop of nutrient medium they form the archusia and it is retained by them in quantities ample for growth. They grow independently. Such stagnant narrow confines are found only in cancer and in the stagnant pools where bacteria grow. In the normal organism such conditions also exist in the very earliest periods of development. On account of certain peculiarities of the egg cells and the early formed blastulae imposed on them by their yolk content they cannot form ample archusia for their growth. At a later period the vascular supply is too great for the production of this substance by the cell and its retention in quantities sufficient for growth. The archusia (S) is not specific. It is formed by other cells in nature and that formed by the other cells can induce growth in the body cells. As pointed out in other articles this substance supplied by other cells is recognized as the vitamin B of food.

During the early periods of development the stagnation of the young embryos is ample so that the archusia formed by its cells plus that added is sufficient for the growth of the cells. As the blood vessels increase in number, first one tissue and then another loses its power of growth. The cells to suffer first are the fibroblasts and muscle cells, or those carrying the blood vessels. Eventually the cells of other tissues receiving a greater and greater vascular supply cease to grow. Cellular growth maintains then only in the cells of certain regions, epidermis, nails, hair, bone marrow and sex glands. As one of us has pointed out in other papers (loc. cit.) the maintenance of growth in these latter regions can be related to their peculiar sluggish circulation. The area in front of the nail bed is supplied with blood through large sinusoids. These are not different from those carrying blood to the bone marrow or to the liver during its period of most active growth in embryonic life.

This stopping of a growth of the cells at maturity does not mean a loss of power of growth of the cells. It is the result of the gradual development of the vascular supply or the reduction in the concentration of the archusia about the cells. If one impedes the circulation as in the case of injury and the resulting inflammatory process, growth again intervenes in proportion to the decrease in blood supply. Under the same conditions of impeded blood supply the organs hypertrophy. If the cells are sufficiently crowded and the circulation sufficiently reduced this growth becomes independent or cancer develops.

While the cells of the body cannot grow in a concentration of the archusia below S^3 they suffer differential changes in these lower concentrations and function. Only in a concentration of the archusia below S^3 do the cells of the body in general differentiate and the connective tissue cells lay down intercellular substances. As stated above the matrix of bone, the tendons, the connective tissue fibrils, etc., are only coagulated extracellular proteins (fibrinogen). In high concentrations of the archusia the connective tissue cells digest these extracellular proteins, grow and divide by mitoses. Only in low concentrations of the archusia do they cause the precipitation of these proteins about them and retain them as extracellular fibrils.

Function depends not only on a low concentration of the archusia, but also on a localization of this concentration at one end of the cell. This localization is determined by the arrangement of the blood vessels.

In the accompanying paper it has been shown that the archusia coming from other cells in nature is probably only vitamin B of the food. In studying the processes of differentiation, function and the formation of the matrix of bone and extracellular formation in general and the storage of fat in cells, it was noted that these acts are dependent on the continuous liberation of a soap-like substance by the cell. This substance has been called the ergusia. The cells form this substance only when they grow. Under the influence of the conditions suitable for differentiation, function, the development of extracellular substances and the storage of fat in the body, these same cells in the cultures become exhausted of this soap-like substance in a few hours or days. In this paper it has been possible to show that these cells remain continuously active in the normal organism, because this substance is being continuously supplied to them from the outside in the form of vitamin A in the food. The reason why vitamin A is necessary in the growth of bone and the maintenance of the differentiated systems in general in the body becomes explained, therefore, for the first time by these studies.

Vitamin A as our further data show is not derived from inorganic nature, but is probably only a product of decaying animal and vegetable matter, bacteria overgrowing their stagnant environments in nature. It

is liberated from the protoplasm of these cells, so it can be used by higher animal cells only when these bacteria are broken down through an excessive accumulation of their archusia. It is present as the ergusia in the intercellular substances of higher animals and in their stored fat. It is interesting to note, however, that only a few fats, butter fat, etc., contain it in a form sufficiently mobile for other animals to use it. It may be liberated, however, in such a mobile form from other fats by ultra violet light.

It is not surprising, therefore, that vitamin A should be necessary and should stimulate the growth of the higher animal and should inhibit the growth of bacteria. It is used in the building of extracellular substances, in function, in the storing of fat and in the differentiation of cells. Evidences of life in lower form is the growth of protoplasm. Vitamin A is formed in this growth reaction. Organic reactions as they are seen in the cells are incomplete reactions. According to the Law of Mass Action, the addition of any substance formed in a reaction slows or stops the reaction according to the relative amount added. Vitamin A is used in function, in the laying down of intercellular substances, the storing of fat and differentiation. It must accelerate, therefore, these latter reactions and slow the growth reaction of the cell.

CONCLUSIONS

1. In the previous paper it has been shown that cultures of bacteria in their periods of most active growth supply large quantities of vitamin B, but no vitamin A, and that this vitamin B is probably only the normal growth stimulus of any cell in nature.

2. In this paper it has been shown that vitamin A is supplied by these same cultures of bacteria after they have overgrown the medium and have been destroyed by this overgrowth. This vitamin is needed by the functioning organism to replace a certain soap-like substance or substances lost to the cell during its differentiation and function. Its source is the protoplasm of other cells in nature.

3. Vitamin A is essential for the organism in that it is one of the essential substances used in the building of intercellular substances of the body (the connective tissue fibrils, bone, etc.) in the storage of fat and in the function of tissue cells.

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THE INJECTION OF TESTIS MATERIAL INTO THE RAT AND ITS EFFECT ON SPERMATOGENESIS[1]

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It has been shown by Moore (1924a) that a testis of an adult guinea pig, when placed in the abdomen with all its vascular and nervous connections intact and with its vas deferens in normal condition, undergoes a rapid course of degeneration. Beginning about six days after the operation degeneration is carried to such an extent within three weeks that the contents of the seminiferous tubules are practically all removed through absorption into the circulation. Under such conditions an opposite scrotal testis remains normal. Lawrence (1926) has confirmed and extended these results.

McCartney (1923) has studied the effects of injection of spermatozoa into male and female rats and hens. He concluded from breeding experiments, first, that female rats injected with spermatozoa are rendered sterile for periods from two to twenty-two weeks, and second, that hens previously injected with spermatozoa laid infertile eggs for periods ranging from twelve to sixty-seven days after the injections had been stopped. The specific conclusion of McCartney's, however, which has appeared to deserve questioning on the basis of work done in this laboratory, is that "subcutaneous injection of spermatozoa in adult male rats tends to cause destruction of the spermatozoa and atrophy of the testis, which effect may be only temporary." Thus, McCartney believed that he was dealing with the effects of antibody formation in the animal against spermatozoa (a spermatotoxin formation), which caused atrophy of the testis. His conclusions were drawn from macroscopic observations on six animals, no attempt being made to examine the histology of the experimental testes or to check the results by breeding experiments. Guyer (1922) has carried out somewhat similar work on the effects of injection of spermatozoa on the "host" animal, and among other things he concludes that "the spermatozoa of a rabbit which has been repeatedly injected with its own semen are much less viable, both in normal serum and in spermatotoxic serum, than are normal

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spermatozoa." Further, he states that "presumably, such spermatozoa have been influenced in vivo by the spermatotoxic serum of their host."

In the light of the experiments conducted in this laboratory it was difficult to account for McCartney's contention that the incorporation of spermatozoa or testis material into the body of an animal leads to degeneration of the testes. It thus appeared desirable to study further the effects on the testes of injecting spermatozoa and testis material into normal adult males. Due not only to the uncertainty of the results of previous work on this subject but also to the lack of positive evidence for sterility in spermatozoa-injected male rats, together with the fact that such an investigation has no little bearing on the problems of sex research being conducted in this laboratory, it was decided to carry out more extensive experiments on the injection of spermatozoa and testis material into adult male rats, with a subsequent histological study of recovered testis tissue coupled with actual breeding experiments (Quick, 1926). No attempt was made to study antibody reactions, as such, as revealed by agglutination experiments.

The purpose, then, of this investigation was to determine whether the injection of spermatozoa or testis material in male rats has any specific effect on the testis or spermatozoa detectable histologically or by breeding experiments. For suggestion of the problem and for advice throughout its pursuance the writer is deeply indebted to Dr. Carl R. Moore.

Material and methods. White rats were used throughout these experiments, both as experimental animals and for obtaining the injection material. Two types of material were used for injection purposes, *a*, spermatozoon suspensions, and *b*, suspensions of macerated testes. The spermatozoon suspensions were prepared by finely cutting and subsequently grinding in a mortar two epididymi from an adult breeding rat and adding approximately 5 cc. of a warmed physiological salt solution. Filtering through a single layer of gauze removed all large particles and the suspension was introduced subcutaneously or intraperitoneally by a hypodermic syringe. In preparing the testis suspensions the two testes (without epididymi) were finely ground in a mortar and similarly suspended in physiological salt solution, filtered, and injected. It will be apparent that the first preparation was almost a pure suspension of spermatozoa, whereas the second contained fewer of these cells, but also considerable formed material and extractives from the testis. At each injection the animals received either the material from two testes or the spermatozoon content of two epididymi. Table 1 shows that the animals received from 1 to 5 injections of the spermatozoon suspensions or suspensions of macerated testes, the injections being made at three day intervals.

Following the desired number of injections of the selected material, and at certain intervals, the animals were mated to test their fertility. The

testes were removed at chosen times and preserved for histological study. Upon their removal an examination was made to determine the motility of the contained spermatozoa.

For histological examination the excised testes were immersed in an excess of Bouin's fixing fluid for approximately one hour, after which they were sectioned transversely into four or five blocks, and then allowed to fix for twenty to twenty-four hours. At different levels complete transverse sections of the testes (7μ paraffine sections) were stained with hematoxylin-eosin and Mallory's III.

RESULTS. It is well known that foreign material, injected subcutaneously or intraperitoneally, often brings about the formation of cysts or sterile abscesses. Such cysts are usually made up of a central coagulated area or necrotic portion and a capsule-like wall containing various wandering cells. At times, reactions to such injected material may cause multiple adhesions of the intestines or the formation of isolated cysts on different organs. On the basis of such reactions, and for ease of description, the series of injected animals has been arranged into three groups.

Group 1 (see table 1) contains those animals whose peritoneal cavity, testes, epididymi, and scrota were entirely free from cysts or abscesses. Group 2 consisted of two animals which showed cysts or abscesses on the sides of the testes, epididymi, or peritoneal viscera, but their location and size was such that the normal scrotal position of the testes was unchanged. In group 3 are placed all cases where, due to the mechanical effects of cyst formation, an abnormal scrotal position of the testes existed. Such groupings were made because we have found in this laboratory that any abnormal scrotal position of the testis is very injurious to normal testicular activity (Moore, 1924a; Moore and Quick, 1924b; Oslund, 1924).

Referring to table 1 we see that eight animals are included and that they received from one to four injections, both subcutaneously and intraperitoneally, of spermatozoon suspension or testis material. Furthermore, the testes of these animals were removed at from three to one hundred and twenty days after the last injection had been given, and that the matings of these animals occurred at from four to one hundred and twenty days after the last injection. The testes, with the exception of a small part of two, were entirely normal histologically; that is, germ cell differentiation was present in all its normal phases and the usual numbers of spermatozoa were present in the lumina of the tubules. The tubules of the epididymi were filled with spermatozoa, which, on being squeezed out, showed normal activity. All but two of the animals produced normal litters, and these two were mated but once. Recent investigations by Evans and Bishop (1922) have shown that under optimum conditions, out of more than a hundred normal matings approximately 20 per cent are not followed by delivery of young.

TABLE 1

ANIMAL NUMBER	INJECTIONS*	REMOVAL OF TESTES; DAYS AFTER LAST INJECTION*	HISTOLOGICAL CONDITION	REMARKS	COPULATION ON DAYS AFTER LAST INJECTION	LITTER*
Group 1						
12	1 S Sc	R 3	Normal		26th	(+)
		L 90	Normal			
13	1 T I	R 3	Normal			
		L 90	Slight disorganization		26th	(−)
14	3 S Sc	R 3	Slight disorganization			
		L 90	Normal		18th	(+)
16	4 S Sc	R 13	Normal		6th	(+)
		L 120	Normal		103rd	(−)
					120th	(+)
17	4 T I	R 23	Normal	Animal died on 26th day after last injection	7th	(+)
					16th	(−)
19	5 S I	R 18	Normal		8th	(−)
		L 38	Normal			
20	3 S I	R 9	Normal		9th	(+)
		L 28	Normal			
27	4 T I	R 30	Normal		No mating	
		L 30	Normal			
Group 2						
18	4 S I	R 18	Normal		4th	(−)
		L 30	Normal	Cyst on head of epididymis	11th	(+)
21	3 T I	R 6	Normal			
		L 25	Normal	Cyst at side of testis near top	5th	(+)

* Numerals indicate the number of injections; S, sperm suspension; T, testis suspension; Sc, subcutaneous injection; I, intraperitoneal injection. Thus, 5 S I denotes 5 intraperitoneal injections of sperm suspension.

R and L refer to right and left testis respectively.

(+), litter; (−) no litter.

TALE 1—*Concluded*

ANIMAL NUMBER	INJECTIONS*	REMOVAL OF TESTES; DAYS AFTER LAST INJECTION*	HISTOLOGICAL CONDITION	REMARKS	COPULATION ON DAYS AFTER LAST INJECTION	LITTER*
			Group 3			
26	5 T I	R 37	Normal		No mating	
		L 37	Degenerate	Cyst on tail of epididymis		
15	3 T I	R 3	Normal			
		L 90	Degenerate	Cyst on vas deferens	18th	(+)
28	4 T I	R 28	Local degeneration	Two cysts at side and bottom of testis	6th 8th	(+) (+)
		L 28	Local degeneration	Cyst at side of testis		

In the two testes, small areas of which might be considered as slightly abnormal, the lumina of approximately a dozen tubules in the superior portion of each testis contained several spermatids and secondary spermatocytes, due, it is thought, to mechanical injury in handling. Of these two animals, no. 13 and no. 14, no. 13 received but one intraperitoneal injection of testis material while in comparison animal no. 17 received four similar injections and showed no evidence of degeneration. Furthermore, animal no. 14, with three injections, produced a litter from a mating which occurred eighteen days after the last injection. These considerations lead us to believe that the injections neither were the cause of the slight degeneration in animals no. 13 and no. 14, nor did they affect fertility in these animals.

Among the eight animals in group 1, then, there seems to be no clear indication that subcutaneous or intraperitoneal injections of heavy spermatozoon suspensions or the formed material of macerated testes have any effect upon testicular activity. Testes removed from these animals at intervals of from three days to four months after the last of two, three, four, or five injections (at three-day intervals) were found to be normally active in germ cell differentiation; spermatozoa in the epididymi were found to be motile, and all the injected males upon mating produced normal litters with the exception of two which were mated but once.

Histological examination of the testes of the two animals in group 2, removed on the 6th, 18th, 25th and 30th days after the last injection revealed that the activity of the testes had been in no way affected. Cysts

or abscesses had been formed in these cases but were small and not attached to the seminiferous tubule portion of the testes. The spermatogenetic activity within the testes was entirely normal, while examination showed spermatozoa from each epididymis to be actively motile, and litters resulted from matings on the 5th and 11th days after the last injection. It will thus be seen that the mere formation of a cyst is not fundamentally important, but when we consider the animals in group 3 it will be noticed that the *size* of the cyst and its effect in altering the normal testicular environment is of prime importance.

The animals in group 3 are all cases in which relatively large cysts were formed in close relationship with the testes, but as in animal no. 26 the two testes may be differently affected. Killed on the 37th day after the last of five intraperitoneal injections of testis material a large cyst was found on the tail of the left epididymis which had caused the left testis to be pushed upward into the inguinal canal towards the abdomen. There were no cysts on the right testis. Histological examination of the left testis showed it to be highly degenerate; no normal seminiferous tubules were found and the majority contained only fragmenting debris and a few Sertoli cells. The picture is entirely similar to that found in testes purposely displaced into the abdomen. The right testis was entirely normal. This animal was not mated. It is entirely evident, therefore, that the secondary reaction of cyst formation and the consequent displacement of the testis was the cause of degeneration in the left testis rather than the primary effect of absorption of the injected testicular suspension; the right testis, subjected to the same possible influence of absorption of injected material for the same length of time, was unaffected.

Somewhat similar conclusions follow for animals no. 15 and no. 28 in this group. In these animals, as in all others of this series, careful and frequent palpations of the scrotum and abdomen were carried out from the period of the injections until the animals were killed. Such palpations led us to believe, as McCartney has suggested, that some of the testes were undergoing hypertrophy. On autopsy, however, it was revealed that the apparent hypertrophy of the testis was only due to cyst formation in the scrotal sac or on the testis as a reaction to the injected formed material that had passed down through the open inguinal canal. The testes themselves were in no case increased in size. For example: animal no. 26 was recorded throughout a period of three weeks after the last injection as having an extremely hypertrophied left testis. On killing, however, the bottom of the scrotum was found to be indurated and a large cyst had pushed the testis up into the inguinal canal. Thus, the apparent testicular enlargement was in reality an induration and abscess formation on the scrotum, and the left testis, itself, was actually smaller than normal and was degenerate throughout.

Although four of the six testes from these animals showed degeneration in varying degrees, two of the three animals produced litters from matings occurring several days after the last injection. It appears, then, that the fact of prime importance brought out by a study of these results, is that the cysts, when formed in suitable positions, exert a purely mechanical influence on the testes which leads to a displacement from their normal environment. This, as has been pointed out, is disastrous to the part of the testes thus affected.

DISCUSSION. Our primary interest in these experiments has been to determine whether the introduction of spermatozoa or the material of the testis itself into a male rat has any deleterious effect on the activity of the testis. McCartney, in his antibody studies, has indicated that such introductions by injection produces sterility and causes the testis to degenerate after hypertrophy. In previous experiments (Lawrence, 1926; Moore, 1924a; Moore and Quick, 1924b; Oslund 1924) absorption of the entire seminiferous tubule content of one testis (when made cryptorchid) has failed to show any effect on the opposite testis whose position was normal. Since such absorption of material into the blood stream from the degenerate testis failed to produce deleterious effects on the opposite testis, it seemed illogical to suppose that the injection of similar material into the animal under less favorable conditions would bring about such an effect. McCartney's reported observations, though not extensive, state that such conditions follow the injection of spermatozoa.

In considering the results of the present investigation it should be emphasized that only *apparent* enlargements of some of the testes were noted before the animals were killed. I have called attention to the fact that cyst formations in the region of the testes may lead to misconceptions of the conditions actually existing in the scrotum, and autopsy has shown that none of the twenty-five testes examined in this experiment were really enlarged. Furthermore, four testes (no. 26, L; no. 15, L; and no. 28, L and R) definitely noted as "apparently hypertrophied" before autopsy were actually found to be smaller than normal and decidedly degenerate in structure. This was caused by an alteration of the normal scrotal environment either through cysts causing the testis to be displaced upward, or through induration and abscess formation on the internal walls of the scrotum. McCartney has interpreted similar conditions as an hypertrophy followed by atrophy and degeneration due to specific antibody effect of the injected material. It is seen that my observations do not lend confirmation to these opinions. The degeneration of the testes appears without any doubt to have been due to secondary mechanical effects acting on the position of the testes in the scrotum through cyst formation, as a reaction to the injected material, and not to the primary effect of absorption of this material into the blood stream. Microscopic examinations

of the testes at various intervals after the last injection and matings of these injected males, followed by delivery of normal litters, complete the chain of evidence in support of the opinion that there is lack of proof pointing to a *specific* effect of the injected material in producing testicular degeneration.

As a result of the secondary cyst formation, a reaction to the injected material, in producing upward displacement of the testis, we have support from another angle for the idea developed earlier that a normal scrotal environment is absolutely essential for normal testicular activity (Moore, 1924b; Moore and Quick, 1924a). Whenever the testis position has been interfered with, either by actual surgical displacement, enlarging cysts in the scrotum, or by hypertrophied epididymi, (a result of vasectomy procedures), degeneration occurs in proportion to the degree of interference (Moore and Quick, 1924a; 1924b; Oslund, 1924).

SUMMARY OF CONCLUSIONS

1. One to five subcutaneous or intraperitoneal injections of spermatozoon suspensions or macerated testis material into white rats has had no apparent specific effect on the activities of the testis. Testes were found to be normal from three days to four months after the last injection. Spermatozoa from such testes were motile and litters were obtained from matings in a major number of the cases.
2. The injection of formed materials resulted in the formation of cysts in some cases, the size and position of some of which brought about mechanical displacement of the testes from their normal scrotal environment. Degeneration of these testes then occurred. Such conditions are not primarily related to the absorption of material into the blood stream of the animals.
3. The scrotal position of the testis is again emphasized as being essential for the continuance of normal testicular activity.

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THE ACTIVITY OF DISPLACED TESTES AND ITS BEARING ON THE PROBLEM OF THE FUNCTION OF THE SCROTUM[1]

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In the course of work in this laboratory on the mammalian testis it has been disclosed that the scrotum functions to regulate the temperature for the testes, and that spermatogenetic activity is dependent upon this function (Moore, 1924b, c, d, 1926a; Moore and Quick, 1924; Moore and Oslund, 1924). Without reviewing in detail all the contributing elements that serve as proof for this conception, one or two particular phases should be recalled for the bearing they have on the present report of experiments.

We have studied in some detail the reactions in a testis made artificially cryptorchid through elevation from the scrotum into the abdomen; under such conditions the vascular and nerve supply, as well as the ductus deferens, remain normal. The testis may be confined to the abdomen permanently by suturing it to the internal body wall or by closing the inguinal canals. But in approximately 50 per cent of cases a testis merely loosened from its scrotal sac attachments and turned back through the open inguinal canals into the abdomen will so remain (guinea pig). Such testes as remain confined to the abdomen become highly disorganized within a period of less than a week; the germinal epithelium begins to slough into the lumen of the seminiferous tubule, or to be liquefied in situ, and absorbed. Within a period of three weeks after elevation, or earlier, the testis will have been deprived of all its germinal epithelium with the exception of spermatogonia (greater degeneration in guinea pig than in the rat). Such testes become progressively smaller with time; seminiferous tubules shrink in diameter, and cell division in the tubules is seldom found. If, however, a testis so elevated to the abdomen is later found to have returned to the scrotum it may be wholly degenerate, partially degenerate, or normal, depending upon the length of abdominal retention, its degree of degeneration before scrotal return, and the length of time since its redescent. A guinea pig testis retained in the abdomen for 20 to 30 days, and showing a loss of all germinal cells but a few spermatogonia, was returned

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to the scrotum (by operation) with almost complete recovery three and one-half months later; a large majority of the seminiferous tubules showed all stages of germ cell production, including spermatozoa (Moore, 1924c).

Such facts have constituted one line of evidence pointing to the scrotal thermoregulation for the testes. The higher abdominal temperature (Moore and Quick, 1924) is considered to be the primary cause of testicular degeneration. By other experimental procedures we have demonstrated, conclusively it seems, that a cooler environment than the interior of the abdomen is necessary (in most mammals) for proper testicular function and that the scrotum provides such a necessary environment.

In the guinea pig we have found that testis grafts in a subcutaneous locality will progress further in the differentiation of germ cells than grafts located in the abdomen (Moore, 1921b; 1926a). Such a fact leads one to believe that ordinary skin can to some extent, carry on the heat regulatory function of the scrotum, though apparently to an insufficient extent to permit spermatozoon differentiation.

In order to study further the possibilities of testicular function in an environment regulated only by the unmodified skin, I have performed several operations on prepubertal rats and guinea pigs in which one, or both, testes were removed from the scrotum to a subcutaneous position in the groin. Testes confined to such localities for periods of months have been compared with normal and with abdominally retained testes and have been shown to carry on spermatogenesis in contrast with a lack of such development in the testis in the abdomen. The present paper presents a few details of testis activity underneath the skin in comparison with cryptorchid and normal testes.

Materials and methods. The rat and guinea pig have served as the experimental animals for these observations. Operations, usually done on prepubertal animals, consisted in loosening the testis from its connection with the scrotal sac and transferring it to the new location. An artificial aperture in the dorso-lateral muscular wall permits of carrying the testis, with its attached blood vessels, nerves, and ductus deferens, into a new cavity made by separating the skin and muscle wall. By closing this artificial aperture and the mid-ventral incision the testis remains completely outside the abdominal cavity between the skin and muscle wall on the ventro-lateral side, and yet supplied by its normal vascular channels.

For comparison the opposite testis was usually freed from the scrotum and elevated to the abdominal cavity, or it may have been left undisturbed; in some animals both testes were placed subcutaneously.

The animals were sacrificed at different periods after operation for histological study, in which entire cross sections of the testes were employed.

Observations. Gross observations at autopsy have been supplemented by an histological study of the recovered tissue. Usually the sub-

cutaneous testis is found tightly adherent to, and covered by, the fascia of the abdominal muscles. Under such conditions the organ is distorted and flattened, but clearly distinguishable from the muscle wall to which it is adherent. At other times, however, a sufficient amount of subcutaneous fat has been deposited about the testis to prevent its being surrounded by the stronger fascia and subjected to the deforming pressure. Under such conditions the characteristic outline had been retained and though it was usually smaller than a scrotal testis of similar age, it was apparent that considerable increase in size had followed its removal to the subcutaneous position. Compared with a testis retained for a similar period in the abdomen, it was usually larger. The new canal, through which passed the ductus deferens and blood vessels, was easily located; the blood supply had suffered no apparent interference and there was no tendency for hernial protrusions.

Histological study shows that whereas the testis firmly bound to the muscle wall and distorted in shape is in a highly degenerate condition, those testes less constricted by fibrous tissue and slightly protected by surrounding fatty tissue are to be found in a high degree of spermatogenetic activity. In so far as the distorted, constricted, degenerate testes in subcutaneous areas are concerned there appears to be nothing of any special significance. Such testes are degenerate but differ in no material way from other degenerations that have been described; the structure of the seminiferous tubules is similar to that shown in figure 2B.

In contrast to this degenerate type, however, those testes better protected from undue fascial pressure are of particular importance in so far as they show, at least to some extent, the amount of differentiation possible in a position of this character. One or two cases will serve to illustrate some of the details observed.

Rat. Animal 236, age 65 days. Each testis freed from scrotum. Right carried through aperture in body wall to subcutaneous position in groin. Left testis with ductus deferens attached turned back into abdomen; left inguinal canal closed; right open. Animal killed 96 days after operation.

At autopsy the right testis was found to be surrounded by considerable subcutaneous fat in the groin and thus not subject to the deforming pressures of the deep fascia; the skin elevation clearly marked its location before killing the animal. It was smaller than similar aged scrotal testes and in this animal it was to have been compared in size and structure with the opposite testis confined to the abdomen for a similar period. But, as rarely happens, the left (abdominal) testis had in some manner become displaced towards the right side of the animal and had descended through the open inguinal canal into the scrotal pouch of the right side, the left canal having been closed at operation. The left testis was of normal size, de-

spite its having been displaced into the abdomen for an unknown period of time; it was for this peculiar condition that this animal was chosen to illustrate the different activity of the two testes.

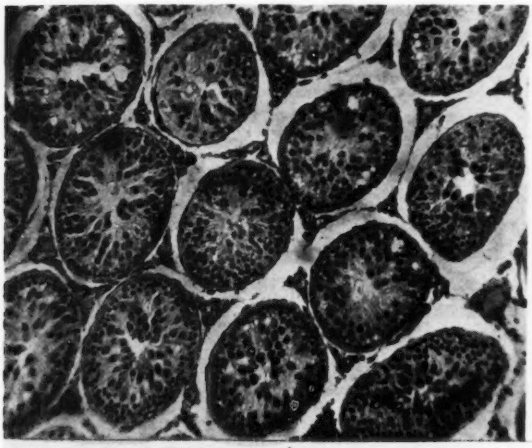
Figure 1, a photograph of tubules from the right testis, can serve to represent the type of seminiferous tubules existing in the testis after remaining in the subcutaneous position for a period longer than three months. The majority of the tubules contain an active germinal epithelium of two to three cells in thickness. Spermatogonial and spermatocyte division figures are numerous and sometimes cells are present that have been questionably interpreted as spermatids; no spermatozoa have been seen.

Many of the spermatocytes are noted as atypical; the cells are often larger than is characteristic for normal testes and various stages of cell degeneration are evident. The atypical character of the cells makes it difficult to be positive in identifying spermatids. It can be seen many times that spermatocytes with nuclei in the spindle stage of mitosis are located in the lumen of the tubules or in other ways indicate a process of degeneration; fragmented remains of such cells show without doubt that they do undergo degeneration.

The left testis of this animal was elevated to the abdomen to serve as a comparison with the subcutaneous testis, but due to its descent into the scrotal pouch of the opposite side subsequent to operation, it was found to be entirely normal, and it contained quantities of spermatozoa. Comparison with testes of other animals having had a somewhat similar period of confinement in the abdomen, however, shows that a greater differentiation has taken place in the subcutaneous testis than in the abdominal one. It has been mentioned before that the rat testis usually suffers less from abdominal confinement than does the testis of the guinea pig, due supposedly to a thicker skin and abdominal wall in the guinea pig and a larger amount of viscera; it has been noted that thermometer readings in the rat abdomen are more variable, and subject to a wider range than is that within the abdomen of the guinea pig.

It is evident, therefore, that a testis removed from the scrotum and placed underneath the skin will carry on its spermatogenetic activity for a considerable period of time (here three months). Normal progress appears to obtain up to and including the spermatocyte stage of differentiation. This period appears to be a relatively critical one in the process of germ cell production and progress beyond this point, if occurring at all in this environment, is the exceptional thing. Spermatocytes or their products (spermatids) escape from the germinal epithelium and are destroyed, with the result that the spermatozoon stage of development is not attained.

The differential effectiveness of the subcutaneous and abdominal environment is more clearly and forcibly demonstrated by a study of the effects of similar operations on guinea pigs, especially when considerably



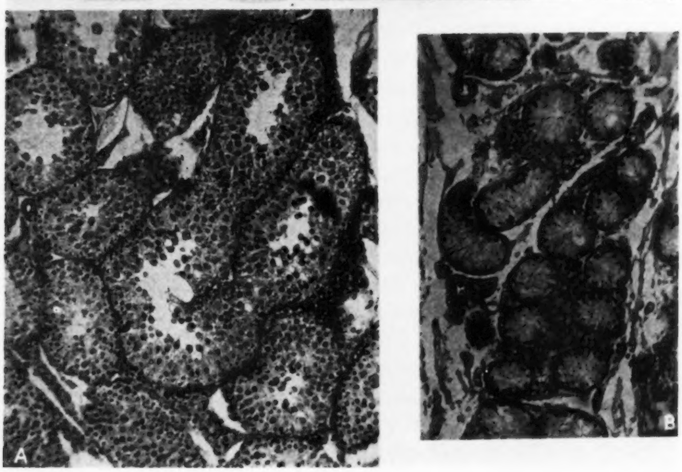


Fig. 1. Photomicrograph of seminiferous tubules from a rat testis (173A) located in a subcutaneous position for three months; spermatogenesis continues but is never complete.

Fig. 2A. Photomicrograph[2] of seminiferous tubules from a guinea pig testis (109B) located in a subcutaneous position for nine months; germinal epithelium continues active but spermatozoa are never produced.

2B. Photomicrograph of seminiferous tubules from a guinea pig testis (109A—partner of fig. 2A) located in the abdomen for nine months; no germinal epithelium activity.

[2] Photomicrographs made by Kenji Toda.

longer post-operative periods are allowed to elapse before recovery of the testes.

Guinea pig. February 25, 1924—male guinea pig 193, 30 days old. Right testis removed from scrotum; carried to subcutaneous position anterior and lateral to scrotum, ductus deferens, vascular supply uninterrupted. Left testis elevated to abdomen, inguinal canals closed. November 28, animal killed (9 months after operation).

At autopsy the left testis was found in the abdomen, without adhesions or twisting of ductus deferens or blood vessels.

A decided skin elevation slightly anterior to the rudimentary mammae marked the subcutaneous position of the right testis. It was surrounded by subcutaneous fatty tissue in the anterior part of the groin and since it was not firmly bound down to the muscle wall by fascia the characteristic shape of the organ was retained. In size it was smaller than a similar aged scrotal testis but was more than double the size of its partner located in the abdomen. The vascular supply and ductus deferens were readily seen to penetrate the lateral abdominal wall and were followed uninterruptedly to their internal connections. There was no evidence that the elevated skin over the subcutaneous testis was thinner than normal.

Figure 2A shows the character of seminiferous tubule differentiation in the subcutaneous testis and should be compared with figure 2B, which shows the differentiation existing in the tubules of the opposite testis located in the abdomen for the same length of time. In the subcutaneous testis, here as in the rat, spermatogenesis has continued for months without culminating in the formation of spermatozoa. In this case more than nine months have elapsed since the operation. But, again as in the rat, there is considerable uncertainty in definitely deciding that differentiation has resulted in the production of spermatids; no spermatozoa have been found in the tubules of this or of any subcutaneous testis. Lack of spermatid or spermatozoon differentiation is not due to a less active division of the earlier cells of the germinal line as quantities of dividing spermatogonia and spermatocytes are present. We have evidently to deal with an external rather than with an internal factor to explain this condition and it is believed that this restricts itself to the factor of the environment.

The partner (left) testis residing in the abdomen (see fig. 2B) consists of tubules entirely quiescent. No dividing cells are present; the tubules are markedly diminished in size compared with the subcutaneous or a normal testis; and they contain apparently only the Sertoli syncytium though an occasional spermatogonium may be identified. The abdomen is, therefore, a less effective environment for spermatogenesis than is the region immediately underneath the skin. This is interpreted, in the light of other work, as indicating that normal skin may be somewhat effective in heat regulation but considerably less so than is the scrotum covered by its modified skin.

These results should dispel any inclination to believe that the degenerate abdominal testis is so merely because of the operative manipulations. Removing the testis from the scrotum, passing it through the body wall, and placing it in a subcutaneous location is many times more severe than is mere abdominal elevation. But despite this fact it is the subcutaneous testis that carries on its spermatogenetic activity for nine months, whereas the abdominal testis shows no progress in this direction, and instead is entirely quiescent in germ cell activity. It appears unquestionably to be an environmental factor that has to be considered.

Discussion. An examination of testicular activity after displacement from its normal scrotal environment is the primary interest in our observations here reported. It has been previously shown that the scrotal environment is necessary for complete spermatozoon differentiation (Moore, 1924a, b, c, 1926b). But testis grafts have in many cases, especially in the guinea pig, shown greater capacity to complete germ cell differentiation underneath the skin than in the abdomen. And having recognized different grades in effectiveness in germ cell production in localities which were not entirely the equivalent of the normal scrotal relationship, it was desired to test the possibility of such differentiation in a testis that has never been separated from its normal blood supply, or exposed to possible chemical differences by transplanting it from one animal to another.

In transposing the testis from its scrotal environment through the body wall to a subcutaneous position, it is difficult to prevent excessive adhesion to the muscle wall. Being continually pressed against the injured fascial covering of the abdominal muscles by the skin, more often than not the testis becomes surrounded by the heavier fascial layers and is firmly bound against the muscle wall. Such deformed, constricted testes are almost always highly degenerate. Occasionally, however, fatty tissue developing between the testis and the abdominal wall provides an insulation within which the organ may retain its characteristic shape. In the latter testes differentiation of the germinal epithelium progresses much beyond those closely adherent and deformed under excessive pressure.

Concerning the activity of testes in subcutaneous locations it is not apparent that spermatogenesis has been markedly interfered with until the spermatocyte stage is reached. Spermatogonial and spermatocyte divisions are numerous and the cells are characteristic but the important consideration is that despite the activity of the germinal epithelium the testis does not acquire the final cells of the germinal line (spermatozoa), even within nine months after operation (guinea pig). Conditions are not such as to permit the progress of spermatogenesis to reach its final culmination. The germinal cell line ends with sloughing of cells into the lumen of the tubules, there, or in the epithelial wall, to undergo degeneration.

Contrast such a condition of the subcutaneous testis with that of the

partner testis removed from the scrotum at the same time, but placed in the abdomen instead of between the abdominal wall and skin. In the latter case there is no sign of spermatogenesis. I have shown earlier that normal adult testes similarily elevated to the abdomen begin a process of degeneration and dissolution of the germinal epithelium within five or six days, that is complete usually in two or three weeks (Moore, 1924c). Such testes never again recover their spermatogenetic powers unless of their own accord, or by operation, they are again brought into the scrotum or sufficiently near it to come under its influence.

The collective evidence appears very convincing not only that the capacity for germ cell production is dependent upon the external environment of the testis, but also that the environmental factor is one of temperature regulation. Such a function of temperature regulation, on the basis of many distinct lines of evidence, has been attributed to the scrotum (Moore, 1924b, 1926a; Moore and Quick, 1924; Moore and Oslund, 1924).

The observations presented here are interpretable upon the above basis and are considered as additional and confirmative evidence for the correctness of the conception. We have pointed out before that the temperature of the deeper visceral portion of the abdomen is higher than that immediately in contact with the internal face of the abdominal wall (Moore and Quick, 1924). Though direct temperature readings have not been made in subcutaneous areas it should be perfectly evident that as one approaches the external surface of the animal the gradient of temperature would be downward. Underneath the skin therefore a sufficient reduction in temperature has occurred that the testis is able to carry on its function only up to a certain point. We have described many similar conditions in testes that have only partly redescended into the scrotum after abdominal elevation. Likewise, in testis grafts of both the guinea pig and the rat seminiferous tubules have shown great activity up to a certain point. More complete differentiation was prevented by the fact that cells bounding the lumen became loosened from it, escaped into the lumen and degenerated. I have shown, however, that a testis graft *in the scrotum* will complete its germinal differentiation and produce spermatozoa (Moore, 1924a; 1926b).

It is apparent, therefore, that despite the more severe operative manipulation in placing the testis underneath the skin and outside the abdomen than in merely elevating and confining it within the abdomen, the former testis will continue its gametogenetic function, whereas the latter does not. Certainly the difference lies in the existence of external rather than in internal factors. And with other evidence it would appear very plausible to assign this difference to one of temperature. The unmodified skin can serve as a partial temperature regulator for the testis but it requires the highly specialized region of the scrotum to bring this regulation to an effective point.

It is of interest to compare the condition of differentiation in such subcutaneous testes, whose blood supply and ductus deferens have never been interrupted and which have not been subjected to possible deleterious influences of chemical differences existing in another animal, with testis grafts in similar localities. Such testis grafts as I have described were obtained by transplanting an immature testis from one individual to another, usually older, and often unrelated animal. In the first case, in addition to a lack of blood supply interruption, we are dealing with a testis in the original animal body of which it is a part; whereas in the latter case it is a foreign testis carried over from another animal. In 1919 I described subcutaneous testis grafts recovered from a guinea pig (a spayed female) whose seminiferous tubule differentiation is essentially the same as that of the subcutaneous testis described above (Moore, 1921b). Likewise, in the rat, testes foreign to a host, transplanted subcutaneously, have progressed as far in differentiation as have the subcutaneous testes considered above. One realizes, therefore, that grafts of testes from another animal reach as high a grade of differentiation as the animal's own testis in the same environment. Furthermore, I have shown that a testis of one animal transplanted into an unrelated older animal will carry on differentiation of the germinal line to completion and produce quantities of spermatozoa *if such grafts are made within the scrotum of the host animal.* It appears, therefore, that the possibility of harmful influences from foreign body fluids has failed to express itself if we consider the differentiation of spermatozoa in a graft as any criterion. The failure of other workers to obtain grafts of mammalian testes producing spermatozoa relates itself to the question of the environment of the graft rather than to the question of harmful influences from foreign body fluids acting upon the graft. Thus the scrotum, functioning to provide a proper environment for spermatozoon differentiation, is seen to be necessary for those animals that have developed such a structure, and we have added evidence that the primary element in this function is the rendering of an environment lower in temperature than that for the body in general.

SUMMARY AND CONCLUSIONS

1. Testes of the rat and guinea pig removed from the scrotum to a subcutaneous location may continue their spermatogenetic function from three to nine months, but spermatozoa are not formed.

2. The partner to the subcutaneous testis that has been located in the abdomen for a similar length of time does not carry on its spermatogenetic function.

3. The conditions regulating this difference are external rather than internal and are considered to be an effect of the environment. In the subcutaneous location a partial reduction of temperature has occurred and to this extent testicular function can be carried on.

4. These results are confirmative evidence that temperature regulation is necessary for the primary function of the testis and emphasize again the function of the scrotum as a heat regulatory mechanism necessary for the production of spermatozoa.

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THE EFFECT OF INSULIN ON DECEREBRATE AND DECAPITATE CATS

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Olmsted and Logan (1923) have stated that the blood sugar in decerebrate cats tends to remain at a high level, or to show a delayed fall, while in decapitate cats the fall to the normal level is fairly rapid. Insulin was found to lower the blood sugar in decapitate cats quite rapidly to the convulsion level and often to below this level, but in decerebrate cats insulin did not seem to lower the blood sugar as readily. Decerebrate cats in which the pituitary body had been removed appeared to behave in these respects more like the decapitate preparations, than those in which the pituitary was left intact. Therefore, they concluded that the pituitary body was responsible for the high blood sugar level. They also noted that the blood sugar could be lowered in decapitate cats to well below the convulsion level without signs of convulsions supervening. From this it was concluded that the convulsions were the result of the action of insulin on the bulbar centres, particularly the respiratory centre.

Bazett, Tychowski and Crowell (1924), working with decerebrate cats which they kept alive for a much longer time than did Olmsted and Logan, found the blood sugar remained high for 24 hours, irrespective of the presence or absence of the pituitary body, and that the hyperglycemia was more closely related to the degree of irritation of the brainstem by blood clot, and to signs of respiratory breakdown, than to other factors. They deny, in short, that the pituitary is responsible for the high blood sugar.

Since Olmsted and Logan's work a large number of experiments in this laboratory on decerebrate and decapitate cats has furnished data which confirm the position of Bazett, Tychowski and Crowell, and contradict Olmsted and Logan's original conclusions.

The majority of the decerebrate preparations were made by the Sherrington guillotine method, the cut being at the level of the anterior corpora quadrigemina. In some of these the pituitary body was carefully picked out of the sella turcica with curved forceps, at the time of decerebration; in others it was left intact; in still others the cats were anesthetized the day before decerebration, and the pituitary removed through a hole

drilled in the roof of the mouth. Some were decerebrated by the method of Pollock and Davis (1923). This consists in trephining through the naso-pharynx, exposing and tying off the basilar artery at the anterior end of the pons. If the carotids are now ligatured the blood supply to the parts of the brain anterior to the ligature is cut off and decerebrate rigidity develops within a few minutes. The pituitary body which receives its blood supply from the Circle of Willis is thus removed from the circulation. The decapitate preparations were made in the usual way, the head being entirely removed. Artificial ventilation at a constant rate was supplied and the temperature was maintained at approximately 37°C.

The samples of blood were drawn directly from the carotid artery into a pipette and the blood sugar estimated by the Hagedorn-Jenson method, using 0.1 cc. of blood. This method has been shown to be sufficiently reliable within fairly narrow limits. In a few of the later experiments 0.2 cc. of blood was used and the sugar estimated by a slightly modified Shaffer-Hartmann method (Olmsted, 1926).

In 9 control decapitate preparations it was found that the blood sugar invariably remained high, 0.25 to 0.3 gm. per cent, for $2\frac{1}{2}$ hours after decapitation. At the end of this time the blood sugar begins to fall and within 5 to 7 hours after decapitation it had reached the normal, 0.11 gm. per cent. Insulin was injected into 26 preparations, while the blood sugar was still high. Within $\frac{1}{2}$ hour the blood sugar began to fall, and 2 hours after insulin, it was below the normal, e.g., 0.07 gm. per cent in all except 3 cases, and in these it was about normal. The so-called convulsive level, 0.045 gm. per cent, was reached in 3 cases within 2 hours after insulin, and in 9 cases within 3 hours. These effects are as Olmsted and Logan observed.

Thirteen decerebrate preparations were observed for a period of 5 to 9 hours following decerebration. Of these 4 were decerebrated by tying off the basilar artery, the rest by the guillotine method. Of the latter group, 2 had the pituitary intact, in 7 it was removed. In 4 of these, hypophysectomy had been performed the day before. A very high initial blood sugar was observed in all these preparations, in one case, 0.425 gm. per cent. This was in general much higher than in the decapitate preparations. Six hours after decerebration only 1 showed a normal percentage of blood sugar, the other 12 ranged from 0.150 to 0.350 gm. per cent, regardless of whether the pituitary was intact or removed. In 3 the blood sugar rose gradually during the 8 hours they were under observations, and in 2 others it remained level above 0.2 per cent. In 3 of these 5 the pituitary had been removed.

After injection of insulin the outstanding feature of 17 preparations so treated (guillotine method, pituitary intact, 4; guillotine method pituitary removed, 9; basilar artery tied, 4) was the extraordinary difficulty we ex-

perienced in lowering the blood sugar enough to produce convulsions. Thus only 5 of the 17 developed typical convulsions, and one of these had to be given two massive doses before convulsions appeared. In only 6 of the 17 decerebrate preparations was the blood sugar brought below 0.06 gm. per cent by the insulin, and 3 of these showed only walking movements and not true convulsions during respiratory crisis. One cat required 3 large doses of insulin to bring the blood sugar to 0.04 gm. per cent.

In regard to Bazett, Tychowski and Crowell's suggestion that the high blood sugar in decerebrate preparations is to be associated with stimulation of the brain stem, and with abnormalities in respiration, we noted the great frequency of respiratory crises in these cats, and furthermore, several autopsies showed clearly that there was a blood clot over the surface of the medulla. In 16 out of 20 control preparations and in 17 of the 22

TABLE 1

NUMBER OF CASES	SYMPTOMS	TIME AFTER INSULIN	BLOOD SUGAR
	Decapitate injected with insulin		
		hours	
17	Typical convulsions	2–5½	0.023–0.058
5	Vigorous scratching and hyperexcitable	2–4	0.045–0.070
1	Stretching only	2¼	
4	Hypersensitive only	3–4	0.057–0.082
2	No symptoms		0.067
	Decapitate controls		
	Observed 7–16 hours		
5	Quiet		
2	Kicked, scratched or stretched, at irregular intervals		

preparations injected with insulin, there were definite signs of respiratory distress, often associated with walking movements, vomiting, convulsions, and a rise in rectal temperature.

Furthermore, our results do not bear out Olmsted and Logan's conclusion that convulsions will not occur in decapitate cats when the blood sugar level falls to 0.045. Table 1 is a summary of our observations on 29 preparations following insulin injection, and on 7 control preparations. It will be noted that the severity of the symptoms is associated with the blood sugar level, convulsions occurring in those cases in which 0.045 gm. per cent was reached. But in several cases it was found that the onset of convulsions was delayed for a considerable period after the blood sugar had fallen to 0.045 gm. per cent, for 3 hours. The character of the convulsions is shown in the following description. Cat 58 became irritable and showed frequent scratch reflexes 4½ hours after insulin. Twenty

minutes later it was markedly hypersensitive, and there appeared walking movements with forelegs, vigorous scratching movements, and sudden violent spasms in which both hind legs were drawn up alongside the body; the forelegs were either rigidly extended or were engaged in a peculiar, rapid, shaking motion; the muscles of the shoulder and neck were rigid; the tail curved forward under the body; the penis was erect; the abdominal muscles were in marked spasm; tail hairs were erect. Between convulsions the cat was quiet, but the slightest touch, and even blowing on its side, would precipitate another spasm. This lasted throughout an hour when concentrated dextrose solution was injected subcutaneously. Following this there were no further convulsions and fifteen minutes later the animal was no longer hypersensitive. A second administration of insulin next day to this same preparation again resulted in hyperexcitability $2\frac{1}{2}$ hours later, and violent convulsions 3 hours after insulin, the same symptoms appearing as on the day previous.

In general the symptoms which may occur after insulin in decapitate preparations are extension of both fore and hind limbs, kicking, walking movements, tremors, muscular twitchings, contraction of neck and shoulder muscles, urination, defecation, erection of the penis and of the hair. It is of interest to note that the only symptom which appeared in a decapitate preparation injected with curare and insulin was erection of the penis, showing the effect on the autonomic system at a time when all the voluntary muscles were completely paralysed. Olmsted and Logan noted the similarity of the convulsion in the decerebrate preparation to asphyxial convulsions. Observations on decapitate cats (Olmsted, 1926) whose ventillation had been lowered or entirely cut off, show that their asphyxial convulsions possess much the same features as their insulin convulsions.

CONCLUSIONS

The usual high blood sugar found in decerebrate cats bears no relation to the presence or absence of the pituitary body. The effect appears to be related to injury of the bulbar centres, probably through blood clot, since autopsy in many cases shows a clot to be present on the medulla, and there is practically always an accompanying respiratory crisis.

Decapitate cats usually have convulsions after insulin as well as decerebrate preparations, if the blood sugar falls to 0.045 mgm. per cent but their onset may be considerably delayed.

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STRONTIUM AS A SOURCE OF ERROR IN BLOOD CALCIUM DETERMINATIONS

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In a recent paper, Swingle and Wenner (1925) report the effects of the oral administration of strontium lactate upon the level of calcium in the blood after parathyroidectomy. They conclude that "strontium acts in two ways: 1, by decreasing the permeability of the gut to calcium excretion so that the normal level of this salt is maintained in the blood and tissues; and 2, by a sedative effect upon the nervous system whereby the excitability of the motor nerves is greatly diminished."

On reading this conclusion, the idea presented itself that since calcium and strontium are so much alike in chemical behavior, any strontium absorbed from the intestine and carried in the blood might be responsible for the apparent rise in calcium. Since the Clark and Collip modification (1925) of Kramer and Tisdall's method (1921) was evidently used for the calcium determinations (Swingle and Rhinhold, 1925) and since strontium oxalate is a relatively insoluble substance, it was decided to investigate the error that the presence of strontium would introduce into a calcium determination.

A solution of strontium chloride was made up to contain 10 mgm. of strontium chloride per 100 cc. The analyses are given in table 1.

The figures show that strontium is not recovered quantitatively. A comparison of the solubilities of the two oxalates explains this point. One hundred parts of water will dissolve 0.0051 part of strontium oxalate but only 0.0006 part of calcium oxalate. The insolubility of the strontium salt is increased in the presence of the excess ammonium oxalate used in the precipitation. The figures in table 1 show that about one-third of the strontium is recovered as the oxalate (calculated as calcium) and is available to introduce an error in the calcium determination.

Positive proof that strontium is able to replace calcium in the blood and thus exert a curative effect upon tetany will be hard to obtain. There is great technical difficulty in the separation and identification of the two

elements in the small amounts in which they would occur in blood.[1] Swingle and Wenner (1925) interpreted their results to mean that the effect of strontium is due to the action of strontium upon the permeability of the intestine for calcium. This cannot be stated as a definite fact until it can be shown that strontium is not absorbed into the blood stream. Earlier work showed that the injection of strontium into animals suffering from tetany cured or prevented the symptoms, though strontium salts were not so efficient as were calcium compounds (Berkeley and Beebe, 1909; Voegtlin and MacCallum, 1910–11; Dragstedt, 1925).

The curative effect of strontium might be explained therefore in two ways. It may be due to the *indirect* action of strontium on the excretion of calcium by rendering the intestine impermeable to the latter, as Swingle and Wenner suggest. Orr, Holt, Wilkins and Boone (1924) have shown that factors increasing the amount of $Ca_3(PO_4)_2$ in the intestine will

TABLE 1

Number of cubic centimeter of 0.01 N potassium permanganate required for		
2 cc. $SrCl_2$ solution containing 10 mgm. of strontium per 100 cc.	2 cc. serum	2 cc. serum + 2 cc. $SrCl_2$ solution
0.382	No. 1. 1.08	No. 1. 1.47
0.382	No. 2. 1.14	No. 2. 1.47
Result = 3.82 mgm. Sr per 100 cc. (calculated as Ca)	No. 1 = 10.8 mgm. Ca per 100 cc.	No. 1 = 10.8 mgm. Ca + 3.9 mgm. Sr per 100 cc.
	No. 2 = 11.4 mgm. Ca per 100 cc.	No. 2 = 11.4 mgm. Ca + 3.3 mgm. Sr per 100 cc.

decrease the blood calcium. As $Sr_3(PO_4)_2$ is much more insoluble than is $Ca_3(PO_4)_2$, the formation of the former may divert the PO_4 ion from the calcium and thus influence favorably its absorption. A determination of the blood phosphorus in such cases might substantiate this point. On the other hand, strontium may have a *direct* effect by replacing calcium in the blood. Calcium would exert a more marked curative action since $Ca_3(PO_4)_2$ is more soluble than $Sr_3(PO_4)_2$ and the amount of the two cations in the blood would depend upon the solubilities of their phosphates (Holt et al., 1925).

[1] While this paper was in press, a paper by Ernst Hodel (1925. Helvetica chim. acta, viii, 514) was called to the attention of the author. Hodel reports a method for determining calcium and strontium in the blood and tissues when both elements are present. Attempts to duplicate Hodel's results are now being made in this laboratory.

SUMMARY

When a solution of strontium was added to blood serum, the figure for calcium as determined by the oxalate precipitation method was increased by about one-third of the strontium present. Until a satisfactory method for the separation of calcium and strontium in the blood is devised, it is impossible to state whether strontium alleviates the symptoms of parathyroid tetany by direct replacement for calcium in the blood, or by rendering the intestinal wall impermeable to calcium.

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CRYPTORCHID TESTES AND TESTICULAR HORMONE PRODUCTION

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It has long been known that cryptorchid testes are aspermatogenic. Descriptions of cryptorchid testes have been uniform in so far that all agree that spermatozoa and spermatids are absent. There is a further general agreement among most workers describing these testes, that spermatocytes and spermatogonia are also absent. Sertoli cells only, of the tubular content, remain. This condition has been found true in artificial as well as in natural cryptorchidism. No satisfactory explanation for the cause of this pathological condition of the testis was offered until quite recently. In a previous paper (18, p. 440) the writer very clearly pointed out that degeneration or failure of development in cryptorchid testes is a result of high temperature. In this paper our information concerning the causes of aspermatogenesis was collected for the first time. A quotation from that paper follows: "The evident factor common to all the above conditions (meat diet, obesity, alcoholism, fever and inflammation, and possibly thyroid feeding) is a rise in temperature which is either local or general. In the case of orchitis, there is a local inflammation with accompanying rise in temperature. In obesity, the accumulation of fat in the cutaneous part of the scrotum in sufficient to cause a rise in temperature within. In meat diet, alcoholism, fever and thyroid feeding there is a general rise in temperature of the body which affects the testis, whether its normal residence is in the body cavity, as in birds, or in a scrotum. Where the rise in temperature is general throughout the body it should affect the ovary as well as the testis and this has been found to be the case, experimentally, in thyroid feeding and in acute alcoholism. The temperature within the abdominal cavity is appreciably higher than that within the scrotum and it is probable that this is a factor causing degeneration of the germinal epithelium." Further proof of that theory soon followed in papers by the writer and other workers (16), (17), (15). This field of investigation was then abandoned in order to attack the problems of regeneration of the germinal epithelium and of testicular hormone production in cryptorchidism. In the following pages are presented the results of an extensive study of the condition of the germinal epithelium in cryptorchid testes,

primarily in reference to hormone production, but also in reference to "regenerative" or spermatogenic possibilities.

A complete review of the literature on cryptorchidism would be out of place in this report. Therefore only a few papers will be referred to other than those that are of immediate concern to the present work. In 1827 Cooper (3) published a book in which he dealt with the problem of cryptorchidism. Goubeaux and Follin (9) in 1855, Godard (8) in 1858, Curling (4) in 1866 and then Griffiths (10) in 1893 followed. These and a few others were interested in the cause of the pathological condition of cryptorchid testes. One school advanced the theory that the retention of the testis and its abnormality were together caused by embryological difficulties or weaknesses. Another school argued that the testis itself was potentially normal, but that it failed to develop while it remained in the abdominal cavity. A third school maintained that development of the testis continued to maturity and that regression then took place. Griffiths stated that the testis of a pup when retained in the abdominal cavity changes little. The central cells only of the tubules disappear. It is important to note that as early as this time (1893) it was known that embryonic germ cells existed in cryptorchid testes.

Ancel and Bouin in a series of papers described cryptorchid testes of pigs, horses, sheep and dogs. They reported that the seminal cells were degenerated, and that the internal face of the membrana propria was represented by a nourishing syncytium which in certain cases continued to secrete actively. In one paper (1, p. 83) they stated that in cryptorchid testes there was complete absence of germ cells, but that Sertoli cells were present. However, in another place (2, p. 281) they stated that in the testis of a six months' pig there were embryonic seminal cells. They were therefore aware of the fact that the germinal epithelium was not absent in all cryptorchid testes. They were more interested in the presence of interstitial cells, which were well represented between the poorly developed tubules. Ganier (6) reported finding a few cells of germinal type (prepuberty cells) in a human cryptorchid testis. Whitehead (22) described cryptorchid testes in which he found one layer of cells in the tubules. These he called Sertoli cells. Hanes (11, p. 345) reports that "in very young cryptorchids, large clear primary sperm cells are seen." He states however, that in cryptorchid testes of adult animals there is no trace of sperm-forming cells and that the basement membranes of the tubules are lined by a single layer of Sertoli cells.

The general fact that cryptorchidism, artificial or natural, results in aspermatogenesis was by this time well established. Most of the investigators thereafter paid little attention to the condition of the germinal epithelium. They usually stated very briefly that only one layer of cells (Sertoli cells) remained in the tubules. Their interest lay in the hyper-

trophy of interstitial cells to which attention had been attracted by the statements of Ancel and Bouin.

A few workers did not slight the tubular tissue in their observations. Loeb (14) very carefully described cryptorchid testes of an adult guinea pig, quotations from which follow. "The testicle tubules were lined by one layer of epithelial cells, the outline of which was not very definite. . . . . Occasionally the nucleolus divided into two parts. In various places the tubules were lined with several rows. . . . . Mitoses were seen quite frequently in these tubule cells and monasters as well as diasters were found. . . . . We were only rarely able to find mitoses in the interstitial cells. In this respect the latter differed from the tubule cells in which mitoses were quite frequent" (p. 34 and 35). In another place (p. 39) he states that "Spermatogonia are completely absent; the tubules consist entirely of Sertoli cells." Faure (7) reported that in ectopic testes of the bat the seminal tubules have two types of cells. There are large cells of poor nuclear volume, that have a nucleolus. These cells are along the tubular margin and represent male ovules. Other cells which are smaller, with elongated nuclei and an abundant cytoplasm with ill defined border are mother cells of spermatogonia. Many investigators have reported upon artificial cryptorchidism. Inasmuch as differences exist between natural and artificially induced cryptorchidism their work will be referred to at another time.

Most investigators reporting upon studies of cryptorchid testes have been impressed with the pathological conditions found and have emphasized that side of the work. In spite of that emphasis it has seemed probable that some germinal epithelium, at least in an undeveloped stage, persists. This probability is much strengthened by a close analysis of the literature and this brief review has been given to show that in some of these testes the presence of cells of germinal epithelial origin has been recognized by many workers.

This paper is intended to be a report of a study made of natural cryptorchid testes taken from man, dog, pig and sheep.[1] Only testes found in the abdominal cavity—not inguinal testes—have been used. Pieces of freshly obtained testes were fixed by immersion in Bouin's fluid. Further preparation for microscopic study has been made in the usual manner (see 19, p. 112). The information gained has been supplemented by a study of early stages of spermatogenesis in artificially produced cryptorchid testes of rats and guinea pigs that have shown recovery after being placed in the scrotum; by a study of testes recovering from exposure to Roentgen rays, and by a study of normally developing testes of young rats. This supplemental material will appear in later papers.

[1] I wish to acknowledge my indebtedness to Dr. K. F. Bascom for the use of slides of cryptorchid testes of sheep and pig which have been included in this study.

Variations in the histological picture of tubules of cryptorchid testes exist. All tubules within a testis are not identical. Likewise cryptorchid testes of young animals differ a little from those of old animals. It is quite natural that investigators who were impressed with the pathological condition of these testes should describe and illustrate the most barren of tubules seen. But a statement to the effect that no germ cells remain in cryptorchid testes implies that all tubules are devoid of both embryonic and mature germ cells. This condition has not been found in those testes examined in this study. Some of the tubules are quite barren, but others contained active epithelial cells.

Figure 1 illustrates the condition found in natural cryptorchid testes. The interstitial cells are abundant. The connective-tissue basement membrane of the tubules does not differ from that of normal testes. It is one cell in thickness and is continuous. Along this basement membrane are scattered "Sertoli" cells. The outlines of these cells are not very definite. Von Ebner (21) states that Sertoli cells can be isolated in physiological salt solution as discrete cells and that in non-functioning tubules they assume the form of cylindrical epithelial cells. Some of these cells have nucleoli divided into two parts. Some of them appear to have fairly well defined walls. Some, though not as large and with a nuclear content not as great in proportion to cytoplasm as spermatogonia, are larger and have more chromatin in their nuclei than do typical Sertoli cells. Some of these cells resemble spermatogonia excepting that the chromatin is more diffuse. This layer of cells is often accompanied by an incomplete second layer. The cells of this layer are irregularly placed and have the appearance of having been derived from the basement layer. In many tubules are other cells scattered throughout the colloid material or occupying a central position as in figure 1.

The cells found in these tubules are active and not degenerated. They divide and increase. Those cells present produce other cells. All of the cells are not alike. They show stages of differentiation, or at least of modification. None of them are like typical Sertoli cells of functioning tubules, but somewhat resemble embryological spermatogonia.

Only a few mitoses have been seen in these tubular cells. A few writers have reported that they have never observed mitoses in Sertoli cells and that these cells divide amitotically. Herxheimer and Hoffman (13) reported that they found Sertoli cells with mitotic figures. Loeb has been quoted above as having seen them with mitotic figures. Dalco (5) states that Sertoli cells and spermatogonia divide mitotically and synchronously. The division phase or period in Sertoli cells is probably very short. The type of division seems uncertain, but the fact that division takes place is clearly shown by the cells produced. The cells found in the central part of the tubules are products of the cells found along the basement membrane,

Sertoli cells of normal testes develop from the germinal epithelium. Hague (12, p. 137) states that Sertoli cells probably develop from spermatogonia. Smith (20) was unable to distinguish spermatogonia from sustentacular cells before the latter had become differentiated and stated that Sertoli cells were likely derived from the basal germ cell layer. This statement is probably correct. The writer has found that in testes of rats fourteen days of age the Sertoli cells are not yet differentiated. Soon after this Sertoli cells make their appearance. They develop from indifferent cells that line the walls of the tubules. They change in structure

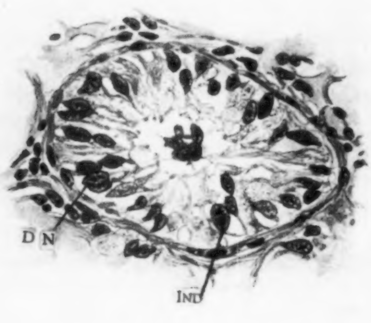

Fig. 1

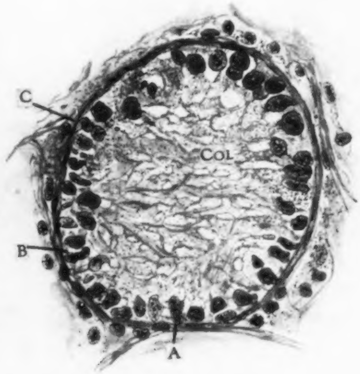

Fig. 2

Fig. 1. Testis M267 of a sheep about nine months old. Note the cells in the lumen of the tubule and other cell of the incomplete second row of cells moving centralward.

Fig. 2. Testis 26R of a dog at least two years old. Note the number of "Sertoli" cells with two nucleoli. *Col.*, colloid which is formed at least in part by disintegration of tubular cells. *A*, *B* and *C* appear to be stages in division of indifferent cells. *Ind.*, indifferent cell; *D.N.*, cell with divided nucleolus.

with approaching spermatogenesis. Spermatogonia likewise develop from indifferent cells. Sertoli cells and spermatogonia are therefore differentiated cells which develop from common mother cells. (From supplemental studies referred to above.) Sertoli cells are therefore cells that develop from the primitive germinal epithelium.

The definitive structure of Sertoli cells in the normal testis is quite different from "Sertoli" cells of cryptorchid testes. In normal testes Sertoli cells are enlarged cells with abundant cytoplasm which forms a syncytium in which are found nucleoli and small pear-shaped nuclei, poor

in chromatin material. In cryptorchid testes, as has been stated above, they are cylindrical cells which closely resemble primitive spermatogonia.

"Sertoli" cells of cryptorchid testes not only resemble the indifferent cells of immature testes, but it seems probable that they are indifferent cells, for when such testes are placed in the scrotum both normal Sertoli cells and spermatogonia develop from them. A series of such experiments will be given later in another paper concerned with the limits of ability of testes to regenerate. Spermatogonia develop from this same type of cell in testes that have been exposed to Roentgen rays. The term *Sertoli cell* should therefore be used to include only the differentiated cells while the undifferentiated cells which produce both Sertoli cells and spermatogonia should be designated as *indifferent cells.*

These indifferent cells are germ cells. Spermatogonia develop from them in maturing testes. Spermatogonia develop from them in testes recovering from exposure to Roentgen rays. This has also been found true in testes recovering from cryptorchidism when they have been placed in the scrotum. The fact that cryptorchid testes are capable of spermatogenesis when placed in the scrotum is strong proof that germ cells are present in these testes.

The misconception concerning the embryonic potentialities of the cells found in the tubules of cryptorchid testes figures prominently in the interstitial cell secretory theory of Ancel and Bouin. They state that germ cells are completely absent from cryptorchid testes and that the interstitial cells are hypertrophied. They then argue that since the germ cells are completely absent and since the interstitial cells alone develop normally the latter must produce the testicular hormone with which these animals are supplied. Their work was based upon observations of natural cryptorchid testes and their statements should therefore be comparable with findings herewith reported.

It has been shown above that the cells found in tubules of cryptorchid testes are active and produce other cells. Some of them resemble primitive spermatogonia more than they do Sertoli cells. It has also been shown that spermatogonia develop from these cells. It is therefore evident that germ cells are present in natural cryptorchid testes and that these cells are active and undergo division. This is contrary to most of the statements found in the literature.

The most that can be said concerning hormone production in these testes is that it is produced during the absence of mature spermatogonia, spermatocytes, spermatids and spermatozoa. From a study of this material it cannot be determined whether the germinal epithelium or the interstitial cells produce the testicular hormone.

SUMMARY

Natural cryptorchid testes of man, dog, pig and sheep were studied with reference to their spermatogenic powers and testicular hormone production. It has been found that the cells in seminiferous tubules of cryptorchid testes are active and produce other cells. These form a second incomplete row of cells or push centralward in the tubules. Some of the cells resemble primitive spermatogonia. These cells produce spermatogonia in cryptorchid testes which show spermatogenic activity after placement in the scrotom. It is quite evident that germ cells are present in natural cryptorchid testes and that these cells are not only potentially active but actually undergo division.

Contrary to most of the reports found in the literature, it therefore appears that germ cells are usually present in cryptorchid testes. Since cryptorchid testes have active germ cells they should produce sperm following surgical transfer to the scrotum. The most that can be said concerning hormone production in these testes is that it is produced during the absence of mature spermatogonia, spermatocytes, spermatids and spermatozoa.

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LIGATION OF VASA EFFERENTIA IN RATS[1]

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In previous papers (1), (3) it has been shown that simple vasectomy does not cause degeneration of seminiferous epithelium in mammalian testes. Likewise it has been shown that vasectomy does not cause a change in the interstitial cells (2). These findings are in accord with much of the literature though in disagreement with the statements of some authors. A review of the literature with an explanation of the discrepancies is given in the papers cited above.

In further pursuing this line of investigation, ligation of the vasa efferentia has been tried. It is intended to present in this paper the results of such a series of experiments together with an interpretation of them and to review the literature on the subject.

The effects produced by ligation of the vasa efferentia depend in part upon the duration of the experiment. During the first one to seven days the testis hardens and increases much in volume. Thereafter, the hardness and size usually decrease. The testis gradually atrophies until it may be no more than one-tenth of the original size. The increase in hardness and size is the result of accumulation of testicular products. The pressure within the testis in some cases increases to such an extent that the testicular walls are ruptured and the contents escape in the scrotum. The decrease in hardness and size results from atrophy and absorption of spermatogenic tissue.

A series of microscopic changes takes place in the tissue structure of the testis paralleling these gross changes. During the first two to four days there is a marked accumulation of spermatogenic cells and of a colloidal material within the seminiferous tubules. They become distended with this material. One week after ligation the contents of the seminiferous tubules become more disorganized. Degeneration of spermatogenic tissue is also seen. This disorganization continues so that within three weeks the testicular material is so disarranged that it may bear little resemblance to its normal structure. Some tubules are full of cellular debris while others contain colloidal material. Some tubules are almost devoid of material

[1] This work was aided by a grant from the Committee on Sex Research of the National Research Council.

other than a scattering of cells along or near the basement membrane. Such a testis may be described as having suffered almost complete degeneration of spermatogenic cells.

While the seminiferous tubules are enlarging from accumulation of spermatogenic material the intertubular spaces are decreasing slightly. The tubules seem to become more closely associated and the intertubular material as a result more compact. When degeneration of spermatogenic cells takes place the seminiferous tubules decrease in size. The entire testis is thereby somewhat reduced in volume but not in proportion to the volume of the tubular decrease. The intertubular space increases considerably, at least during the early stages of the degeneration. This intertubular space is largely filled with lymph. No observable change in the interstitial cells has been found in these experiments.

Material and technique. Albino rats have been used throughout these experiments, partly because of the ease of operative procedure and partly because of the fact that they are adapted to laboratory work. Their testes show normal activity during laboratory confinement and they are reasonably resistant to infection. In rats the vasa efferentia form a fairly isolated portion of tissue leaving the testis adjacent to the spermatic vessels. Care was taken not to injure the spermatic artery or vein and at the same time to occlude all vasa efferentia. Inconstant results are obtained from failure to make the ligation complete. When ligation is incomplete some degeneration takes place. This is followed by gradual recovery. The uninjured tubules apparently carry away the accumulated debris. No attempt has been made to isolate or otherwise protect against the inclusion of the nerve elements or the lymphatic vessels in the ligation. These factors are, therefore, uncontrolled in these experiments. The work here reported is based upon twenty-five experiments, in addition to controls. The results obtained were so constant that further repetition appeared unnecessary.

All operations were performed aseptically under ether anesthesia. Following simple laparotomy the testis was pulled through the inguinal canal from the scrotum and brought into plain view. A silk ligature was carefully placed around the vasa efferentia and tightly knotted. The testis was then returned to the peritoneal cavity, pulled back into the scrotum and the incision closed.

Daily observations were kept until the conclusion of the experiments. The testes were palpated and differences in size and hardness noted. After intervals varying from one day to two months the animals were killed. The testes were carefully cut with a razor into sections about $\frac{3}{16}$ of an inch in thickness, to insure rapid fixation, and immersed in Bouin's fluid. Portions of each testis were dehydrated and prepared in the usual manner for microscopic examination. Sections cut 7μ in thickness were stained in

combinations of Delafield's hematoxylin and Congo red, Delafield's hematoxylin and eosin and Delafield's hematoxylin and orange G. All comparative observations were made upon representative sections taken from corresponding regions of the different testes.

EXPERIMENTAL DATA. The first consequence of vasa efferentia ligation is an accumulation of testicular material in the seminiferous tubules. This material at first fills the tubules near the vasa efferentia gradually causing them to be distended. While the spermatic function of the testis remains, these products continue to accumulate in the tubules filling and distending them more and more. The accumulation of material takes place quite rapidly. Within forty-eight hours they produce an increase in the size of the testis and a very noticeable hardness of it. When the vas deferens is ligated there is a similar distention of the epididymis. This accumulated testicular material is produced by the seminiferous tubules. It is composed of cellular elements and of a colloidal mass which resembles the cytoplasm of Sertoli cells in which the spermatids are embedded while undergoing transition into mature sperm.

Sperm are an abundant constituent of this material during this period of ligation. Spermatids are also a common part of this debris. Some of the cells present resemble spermatocytes.

It is usually assumed, though seldom definitely so stated, that the transformation of spermatogonia into primary and secondary spermatocytes and finally into spermatids takes place in one locality, i.e., without actual movement of the cellular elements along the seminiferous tubules. These spermatocyte-like bodies are, however, commonly found in the epididymis of vasectomized and of normal rats. It seems quite probable that they either migrate or are passively moved a short distance along the tubules together with other material.

The quantity of colloidal material varies considerably but it is usually quite abundant. Its origin seems to be from degenerating cells but this is in no wise certain. It probably forms a considerable portion of the normal sperm fluid and passes down the seminiferous tubules to the epididymis with the sperm. Whether it should be considered a secretory or an excretory product has not been determined. Following metamorphosis of spermatids into spermatoza there appears to be an abundance of this debris in the lumen of the tubules.

The arrangement of elements within the seminiferous tubules would lead one to believe that normally there is in them some flow of material. That there is a considerable volume of this mobile material in the tubules is shown by the rapid distention of the testis following ligation of the vasa efferentia.

Spermatogenesis continues during the first two or three days in the major part of the testis. It is the continued spermatogenesis that adds to the

material within the seminiferous tubules thereby distending them and causing the hardening of the testis.

With increase in size of the seminiferous tubules there is a decrease in the volume of intertubular tissue. In a normal testis a small part of the intertubular space is filled with lymph in which are seen islands of connective tissue cells, interstitial cells, blood vessels and nerves. The intertubular liquid disappears with increase in size of the seminiferous tubules. The islands of cellular material (connective tissue, interstitial cells, blood vessels and nerves) become very compact and occupy but little space between the associated tubules. These cell-clusters are so variable in

Fig. 1

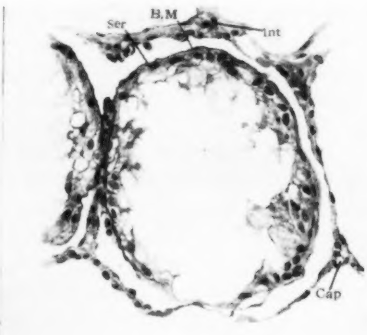

Fig. 2

Fig. 1. Testis of rat 1L. Vasa efferentia ligated 48 hours. This testis was much larger than the right (normal) testis. The mass of debris filling the lumen of such tubules is typical.

Fig. 2. Testis of rat 11L. Vasa efferentia ligated five weeks. The tubule cells are almost completely degenerated—absent. *BM*, basement membrane; *Int.*, interstitial cell; *Ser.*, Sertoli cell; *Cap.*, capillary. Drawings by staff of Division of Illustrations, Department of Anatomy.

quantity that it has seemed impossible to determine whether there has been any decrease in the number of connective tissue or interstitial cells.

The hardening of the testis is but an indication of the pressure of the testicular material within the tunica albuginea. This pressure often becomes great enough to burst this tough membranous covering and even to rupture the walls of the scrotum.

It seems reasonable to suppose that the increased pressure within the testis during this period should have some effect upon the circulation of the blood within. However, when some of the animals were killed physiological salt solution containing methylene blue, when injected into the circulatory system, readily permeated the testis. In the experiments

where the testes were fixed by immersing them in Bouin's fluid, arterioles and capillaries appear well filled with blood. Therefore it appears unlikely that circulation was prevented by the testicular pressure, though it might have been hindered.

In the course of six or seven days marked changes have taken place in the ligated testes. The pressure still exists and the testes are much larger than normal. Sections of such testes show extensive degeneration of spermatogenic cells. The seminiferous tubules contain an abundance of cellular debris like that described above. Some sperm are present. But their number and appearance indicate that less spermatogenesis, if any, is now in progress. Mature sperm are quite resistant to injurious agents and those now found may have been produced during the first few days of ligation. Though there is some variation among tubules within each testis the general picture is one of degeneration. Sperm are no longer found embedded in or leaving the Sertoli cells nor are spermatids present. Even secondary spermatocytes appear wanting. Spermatogenesis is only represented by Sertoli cells, spermatogonia and by some primary spermatocytes.

The tubules do not appear to be distended as before and the intertubular spaces are somewhat larger. As in the normal testis part of this space is occupied by lymph. This was absent in the testes described above in which the tubules were much distended. The quantity of interestitial cells may have increased slightly. Any change in their volume is here negligible.

Experiments of two or three weeks' duration show increased degeneration of germinal epithelium. The testes are generally much reduced in size. Some are about normal in size but hard, while others are less than half normal size and have a peculiar spongy feeling when palpated due to the presence of liquid rather than of solid material. The cellular debris and colloidal material within the tubules have decreased in quantity. It appears that this material has undergone liquefaction and absorption. The seminiferous tubules have decreased in size.

There is much more intertubular space. Most of this space is occupied by lymph, the quantity of which is markedly increased. There is no noticeable interstitial cell hypertrophy and the intertubular cellular elements form island-like masses in the intertubular lymph.

Experiments of four and five weeks' duration show a continuation of the degenerative process. The testes are smaller and full of fluid. In some experiments part of the seminiferous tubules are full of debris, while other tubules are empty. In other experiments all tubules seemed empty. In one experiment of four weeks' duration most of the tubules were full of cellular material and some spermatogenesis was found. It seems probable that in this case some of the vasa efferentia escaped ligation. In all other experiments the seminiferous tubules were almost devoid of sperma-

togenic cells. Immediately adjacent to the basement membrane is a layer of colloidal material. In this is embedded here and there what appear to be representatives of spermatogenic tissue. The tubules have undergone more degeneration than is found in cryptorchid testes. The analogous cells found scattered along the basement membrane in such tubules are described as Sertoli cells.

The seminiferous tubules are somewhat shrunken and the intertubular space increased in size. The cellular constitutents of the latter appear normal in volume; while the remainder of the space is filled with lymph.

Experiments of two months' duration present a picture of more complete degeneration. There are fewer Sertoli cells in the tubules and the tubules have shrunken more. In one experiment the entire testis was less than one-tenth as large as the contralateral normal testis.

Discussion. Ligation of the vasa efferentia produces degeneration of spermatogenic tissue. The seminiferous tubules within the course of one week show marked degenerative changes. Spermatogenesis ceases and the empty tubules shrink in size. The degenerative changes noted increase the longer the experiment is continued. No indication of regeneration has been seen in this series during an observation of two months. The writer has found that it requires three months for seminiferous epithelium to regenerate after extensive degeneration. Regeneration, therefore, could not have been accomplished in these experiments in short of five months. These findings are contrary to those reported by Steinach (5). Results of ligature of the vasa efferentia were reported by that writer as being the same as ligation of the vas deferens. In either case he claimed that regeneration took place in the course of three months. It is hard to understand how regeneration of the germinal epithelium could take place without removal of the ligature. At best, a slight recovery with production of some cells should only produce more injurious debris and the degeneration would again set in. It is quite possible, but it seems highly improbable, nor has it been found true in the experiments herewith reported. Where the ligation has been incomplete, i.e., when some of the vasa efferentia are not included in the ligature the testis first enlarges, but very quickly returns to normal.

Tiedje (6) reported that degeneration results from vasectomy and from ligation of the vasa efferentia. In a former paper (1, p. 435) the writer interpreted these results as accidental cryptorchidism. The three cases in which the vasa efferentia were ligated probably resulted in degeneration as was reported by Tiedje. Either vasa efferentia ligation or cryptorchidism would produce the degeneration.

Degeneration from ligation of the vasa efferentia has been reported by Van Wagenen (8). In her experiments the testes became transparent in twenty-nine days and their volume decreased thirty to fifty per cent. The

germinal epithelium underwent degeneration. It is her belief that blood stasis from pressure results in malnutrition and oxygen want and that degeneration then follows. Aside from details as to time when degeneration first becomes marked our findings are alike.

I have tried to determine by injections and by histological observations whether blood stasis takes place. I am of the opinion that the pressure obtained within the testis must have some effect upon the blood flow. However, I feel sure from injection experiments reported above that circulation within the testis is not greatly hindered.

In reporting some vasectomy experiments Tournade (7) stated that when cysts formed at the point of resection of the vas no degeneration of the testis took place. He suggested that in such experiments the cyst forms a reservoir for the sperm and no increased testicular pressure results. Sand (4, page 495) partly accepts Tournade's theory and interprets the degenerative changes found in his own experiments as a consequence of increased pressure within the testis. Cyst formation was not constant enough however, to explain the results obtained in their experiments. In most cases no degeneration results from vasectomy. In some instances there are found tubules that show a slight amount of degeneration. Only a few tubules are usually affected and the testicular pressure appears not to be increased. The epididymis becomes much distended and very hard. It acts as a reservoir for the products from the seminiferous tubules and thereby relieves the testis proper of this pressure. It is very probable that much of this material is absorbed from the epididymis; some even under normal conditions (9).

When the vasa efferentia are ligated there is no escape for the seminiferous material. The tubules very quickly become filled and distended with debris. Degeneration follows as here reported. Since presence of necrotic material in the seminiferous tubules sometimes causes a slight amount of degeneration in vasectomy experiments without pressure in the testis, it seems probable that it is the important factor in causing the marked degeneration seen in this series. The cellular and colloidal material that is retained by the occlusion of the excurrent ducts is primarily a consequence of the operation. In the absence of a means of escape this material undergoes decomposition. The presence of this toxic debris against the tubular cells soon injures them and degeneration results. This material in the tubules is then secondarily the cause of degeneraton. The resulting liquefied material in the tubules is gradually absorbed, either through the lymph system or via the capillaries into the venules. Whether or not there are germ cells still present that could then regenerate is not certain, but if so, even partial recovery would only be followed by another period of degeneration as suggested above.

Intertubular space varies with the size of the seminiferous tubules.

When these tubules are full they press tightly together. There remains only enough space between them for the cellular elements. The lymph so commonly found in this space is absorbed. In some experiments when there is an abundance of this intertubular lymph the connective tissue cells appear to enlarge and become what is referred to as interstitial cells. Actual proof of such a change would be difficult to obtain, but there have been so many reports indicative of such a change that this transformation seems quite probable (2). In the paper just referred to (2) it was pointed out that interstitial cell hypertrophy does not always result under these conditions. It has not been found in these experiments.

SUMMARY

Ligation of vasa efferentia in rats is followed by an increase in size and hardening of the testis. This is caused by the retention of spermatogenic products in the testis. The process reaches its height in about forty-eight hours. Within seven days the testis decreases in size and in hardness. This change is caused by a decrease in, if not a cessation of, spermatogenesis and by the absorption of the now necrotic material that was retained by the ligature. The process of degeneration progresses with duration of the experiment. Degeneration of germinal epithelium is accompanied by a decrease in the size of the seminiferous tubules. This is partly compensated for by an increase of intertubular space and partly by a decrease in the size of the testis. No interstitial cell hypertrophy has been found. The experiments were followed during a period of two months. Degeneration was still progressing and there was no sign of recovery.

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ON THE ALVEOLAR CO_2 TENSION FOLLOWING VIGOROUS MUSCULAR EXERCISE

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It is now generally recognized that the alveolar CO_2 tension remains low for some time following short periods of vigorous exercise. Douglas and Haldane (1909), working on the influence of muscular exercise on the respiratory center, found that the alveolar CO_2 tension became low several minutes after a rapid ascent of stairs totalling 40 feet in height, and returned to the normal level during the course of about one hour. Krogh and Linhard (1920), studying the respiratory changes during transition from work to rest, observed lowering of CO_2 tension following various intensities and durations of work on a bicycle ergometer. Their observations were made at short intervals varying from 10 to 15 minutes following the exercise. In tracing the development of present views regarding the processes determining the reduction in alveolar CO_2 tension, reference may be made to the work of Ryffel (1909), demonstrating an increase of lactic acid in human blood after vigorous running on the level for about 2 minutes. Christiansen, Douglas and Haldane (1914) found diminished CO_2-combining capacity of blood after exhausting exercise, accompanied by a reduction of one-fifth in alveolar pressure, the altered condition remaining for over an hour. Ryffel's results strengthened their belief in the influence of lactic acid in bringing about the above-mentioned state of affairs. Barr and his collaborators (1923) actually demonstrated increased lactic acid content and diminished CO_2-combining power occurring simultaneously in the same specimens of blood. Haldane and Quastel (1924), using the Haldane method of collecting alveolar air, followed the course of the alveolar CO_2 tension after exercise and reported that the recovery curve (CO_2 tensions plotted against time) is a straight line, i.e., that the CO_2 tension is a linear function of the time.

The present problem had been outlined before the appearance of the paper by the last-mentioned investigators. It was thought, if satisfactory and consistent determinations could be made during the course of the recovery of the alveolar CO_2 tension following exercise, that, not only could

light be obtained on the nature of the process determining the altered CO_2 tension, but also a way might be opened for the study of conditions affecting the behavior of the recovery to the normal tension. Haldane and Quastel's data did not, it was felt, satisfactorily dispose of the problem. Conclusions based on the arbitrary selection from 22 irregular curves, of 10 that are linear, can scarcely be regarded as the best method of interpreting data that are subject to a large experimental error. During the course of the present experiments, it became apparent that account could be taken of the unknown sources of error only by averaging the data of many experiments. When this is done, the recovery curve, as will be seen, turns out definitely to be concave downward. It also became obvious that until ways of controlling the fluctuations could be discovered, the investigation of conditions affecting the recovery curve of alveolar CO_2 tension would prove futile.

The method of collecting alveolar air used was that described by Henderson and Haggard (1925), with some minor alterations. The dead space of the valves and connecting tubes was reduced to a minimum, the total capacity of the system from the mouthpiece to the sampling tube amounting to 10 cc. The latter had a capacity of about 40 cc., and the resistance in the circuit was so adjusted that at each inspiration 15 cc. of the air last expired were drawn into the sampling tube. At the normal rate of respiration, therefore, the air in the sampling tube was more than entirely replaced during the period of collection, and was made up of the last air of several expirations.

With this arrangement normal CO_2 determinations were made on N. C. at regular intervals and compared with the usual pair of Haldane-Priestley samples taken just after each Henderson sample. A reasonably close agreement was found between the determinations by the two methods. A further test of the Henderson method was made by analyzing alveolar air taken at different rates of respiration while the minute volume was kept constant. This was done by inserting a tambour in the circuit between the manometer and the mouthpiece, for registering the respiratory rate, and connecting the inlet tube with a spirometer to register the volume. The Henderson method might be unsatisfactory for two reasons: 1, The dead space might be so large relatively that the air reaching the alveoli might consist in larger part than usual of air already breathed. If this were the case, the effect upon the alveolar air would be similar to that produced by shallow breathing (Haldane, Meakins and Priestley, 1919), namely, a fall of oxygen tension and a rise of CO_2 tension due to the piling up of the latter in the blood. 2, If the air at the end of a normal expiration as sampled by the Henderson method did not come from the alveoli, the sample obtained from a deeper expiration would contain a higher percentage of CO_2. That such is not the case has been proved by the tests

mentioned above. Table 1 shows that doubling the depth of respiration has no effect upon the alveolar CO_2 tension as determined by the Henderson method.

In the present experiments the exercise consisted in running twice down and up three flights of stairs 14 meters in height, at top speed, a performance that doubled the pulse rate and produced marked hyperpnea. After resting for about 10 minutes, or when the breathing had subjectively become easy, samples of alveolar air were collected at certain fixed intervals. In all, 21 experiments were done, 18 on N. C. and 3 on T. H. Collections were made at five- three-, or two-minute intervals for periods varying from

TABLE 1

Deep breathing (minute volume constant) and alveolar CO_2 as determined by the Henderson method of collection

TIME OF TAKING SAMPLE	MINUTE VOLUME (cc.)	RESPIRATION PER MINUTE	AVERAGE VOLUME OF EACH BREATH	CO_2 PER CENT
January 28 (a.m.)				
3:30	6470	14	462	5.37
3:35	6480	7	925	5.55
4:40	6380	12	530	5.45
4:45	6410	7	915	5.56
January 29 (a.m. and p.m.)				
10:00	6400	12	534	5.34
10:05	6530	6	1090	5.35
10:25	6380	12	530	5.72
10:30	6580	6	1096	5.47
10:50	6440	12	535	5.47
10:55	6380	6	1063	5.38
11:30	6580	12	528	5.38
11:35	6400	6	1066	5.45
1:55	6500	14	464	5.43
2:00	6558	6	1093	5.52
2:20	6580	14	470	5.42
2:30	6500	6	1083	5.49

35 to 40 minutes, or until the CO_2 tension had risen almost to the normal level for the particular subject. The most important determinations were those made during the first half hour. Each sample was collected for one full minute. This permitted complete filling of the samplers with a representative sample for that period; the air collected consisted of a mixture of the last portion of a number of natural expirations. Table 2 summarizes the results.

Each individual experiment was plotted and found to show a curvature with the concavity downward, except two instances of linearity. No curve showed concavity upward. Experiments 2, 8 and 12 on N. C. show

TABLE 2

Alveolar carbon dioxide tension following vigorous muscular exercise

EXPERIMENT NUMBER	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Av.	19	20	21	Av.
Date	11/21	11/21	11/23	11/25	11/28	11/28	12/2	12/2	12/4	12/5	12/5	12/12	12/14	12/18	12/18	12/19	12/26	12/28	N.C. 14	1/4	1/7	1/8	T.H. 17
Minutes from end of experiment to 0 point	25	10	15	10	15	15	15	10	10	15	10	10	10	15	20	20	20	20		20	15	15	
Minutes from 0																							
0	33.0	32.0	33.7	29.6	31.3	31.6	31.9	33.7	31.4	32.4	33.4	30.7	31.8	31.5	31.7	30.5	34.0	33.2	32.1	25.0	27.6	27.9	26.8
2												32.2	32.9	28.8	30.9	30.7	34.9	32.8	31.9	25.6	28.0	29.1	27.3
3				30.9	33.1	32.2	34.1	36.0	32.6	32.0	33.7								33.1				
4												33.0	34.8	30.3	34.3	32.2	34.5	32.8	33.1	26.0	28.4	29.5	28.0
5		33.4																	33.4				
6				35.2	34.4	34.5	34.9	35.4	34.0	35.3	34.6	33.8	34.7	30.9	34.4	31.2	36.1	32.3	34.1	27.3	29.1	30.1	28.8
8												35.2	34.7	31.4	34.3	30.5	37.3	34.4	34.0	28.3	30.1	30.2	29.5
9				35.7	33.4	34.5	36.5	36.4	33.5	35.1	34.0								34.9				
10		34.5	34.1									34.3	36.3	32.6	35.6	32.5	37.2	35.4	34.7	27.8	27.9	30.6	28.8
12	35.7			37.3	32.5	34.5	36.9	36.6	35.8	36.5	35.7	34.8	35.5	32.0	33.1		37.3	34.2	35.2	28.3	27.8	30.1	28.7
14												36.5	36.0	33.3	34.4	32.8	37.8	34.3	35.0	27.8	30.8	31.2	29.9
15		36.9	35.4	36.9	35.2	35.2	37.1	36.0	34.5	37.9	37.0								36.2				
16												35.9	36.0	33.4	34.4	32.9	38.8	36.1	35.4	26.1	30.8	31.9	29.6
17	37.2																		37.2				
18				37.2	34.0	34.7	38.2	37.3	35.8	36.8	37.3		37.2	34.2	34.4	34.3	37.4	36.4	36.1	28.9	31.9	33.1	31.3
20		36.8	37.8									35.9	36.5	32.0	34.5	34.6		36.0	35.5	31.8	32.9	33.5	32.7
21				39.6	35.8	35.8	36.7	37.0	36.3	34.3	38.2								36.7				
22	38.0											36.9	35.3	36.7	32.7	32.3		35.1	35.3	30.4	32.9	33.1	32.1
24				38.6	35.7	35.5	39.8	37.8	34.9	38.4	36.6						38.2	35.8	37.1	30.3	32.7		31.5
25		37.3	38.2																37.7				
26																		35.4	35.4	32.0	32.7	33.7	32.8
27	38.2				38.6	36.2	39.5	39.3	37.6	38.3	38.3								38.2				

28																		36.9	36.9	31.3		33.7	32.0
30		38.6	37.2	39.9	37.8	37.3	38.8	39.3	38.3	37.9	38.4							36.5	38.2	32.3	32.9	34.2	33.1
32	40.0																		40.0	31.4	32.7	33.5	32.5
33				40.2	39.5	38.5	38.7	38.9	39.8	38.8	38.6								39.1				
34																		36.9	36.9	33.8	32.6	32.9	33.1
35		37.5	38.0																37.7				
36																				31.4	33.7	31.5	32.2
37	38.9																		38.9				
38																		36.4	36.4	32.5	32.9	33.1	32.8
40		38.9	40.0																39.5	31.2	35.4		33.3

little irregular fluctuation of the successive determinations, and they, likewise, exhibit the downward concavity.

In most of the experiments there was so much fluctuation between the successive determinations that it seemed desirable to ascertain whether this has occurred also in the experience of others. A search of the literature revealed but few successive alveolar CO_2 determinations at short intervals under constant conditions. These are collected in table 3, together with a series of observations by the writer. The range, it is seen, is so wide as to indicate either that our present methods of collection are not entirely

TABLE 3

Fluctuations of successive determinations of alveolar CO_2 tension by different authors

AUTHORS	DURATION OF OBSERVATIONS	NUMBER OF DETERMINATIONS	INTERVAL BETWEEN COLLECTIONS	CO_2 TENSION FLUCTUATIONS, MM. Hg		METHOD OF COLLECTION EMPLOYED
				Average	Maximum	
Fitzgerald and Haldane (1905), Table VII	12 hrs.	17	15 min.	±1.5	±3.2	Haldane-Priestley
Krogh and Linhard (1917) Table 13, A. K.	Same part of the day presumably	6	About 15 min.	±1.4	±2.3	Krogh
Krogh and Linhard (1917) Table 13, J. L.		6	15 min.	±1.4	±3.5	Krogh
Pearce, 1917, Table 3 column 1	Morning	6	About 15 min.	±1.0	±1.3	Pearce
Pearce, 1917, Table 4 column 1	Afternoon	5		±1.6	±2.0	
Dodds (1921), Table 1, Series C	4 hrs.	5	1 hr.	±0.4	±0.6	Haldane-Priestley
Cordero	½ hr.	8	3 min.	±1.5	±2.2	Henderson

satisfactory, or that there are actually minute-to-minute variations in the alveolar CO_2 tension. However this may be, it would be unjustifiable to discard, as do Haldane and Quastel, all measurements that indicate a scattering of points around the indicated curve. In view of the fluctuations in CO_2 tension, the true form of the recovery curve can be obtained only by performing a number of satisfactory experiments that is sufficient to permit of the elimination of the chance fluctuations by averaging all of the comparable data. This is the method followed here.

In table 2, the beginning of the rise in CO_2 tension in every experiment

has been arbitrarily taken as zero. This zero point, therefore, represents a fixed phase in the recovery process, and not any specified time. In one instance (expt. 1) in which determinations were made every five minutes, the first four CO_2 values were such that it was difficult to determine the zero point. Hence, the four CO_2 values were averaged to give the zero. The corresponding four time intervals from the cessation of exercise were also averaged to give the zero from which the subsequent intervals were

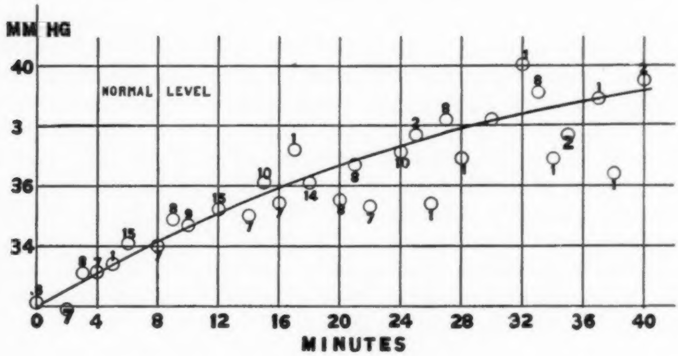

Fig. 1

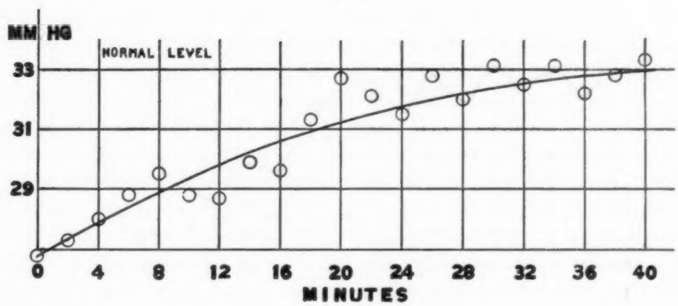

Fig. 2

counted. The result is that many of the subsequent intervals in experiment 1 do not coincide with those in the other experiments. Figure 1 represents the averages in the case of N. C. The figures adjacent to the circles give the number of comparable determinations of which the point is the average. Figure 2 represents the averages of the three experiments done on T. H. The downward concavity of these curves is perfectly obvious.

The lowered CO_2 tension during recovery from muscular exercise, in all

probability, is due mainly, at least, to stimulation of the respiratory center by the increased C_H produced by the lactic acid added to the blood, the resulting increase of lung ventilation washing out the dissolved CO_2. In the present experiments a record was not made of the variations in the volume of the air breathed. The data of Hill, Long and Lupton (1924b) on the oxygen intake following short periods of vigorous muscular exercise showed that the recovery toward normal occurs rapidly during the first few minutes, and thereafter, becomes more gradual during the period of over an hour. Since all of their curves have approximately the same form and duration, they may be taken as a measure of the recovery of the respired volume to normal in our experiments. Now, Hill and his co-workers attribute the first rapid phase of the oxygen recovery curve to the oxidative removal of lactic acid in the muscles, where it was formed; and the protracted phase, to the removal of the lactic acid which has diffused into the blood, owing to the inadequacy of the oxygen supply during and after exercise to remove it immediately. The curve of oxygen intake implies that the ventilation, and hence the blowing off of CO_2 is very rapid at first, and much slower later. Consequently, the CO_2 tension falls rapidly at first, and reaches its minimum level when the overventilation begins to slacken. As this blowing off continues for some time after, though at a much slower rate, the CO_2 tension continues to remain low, but gradually rises to normal. The same general behavior of the tension of CO_2 in the blood following exercise was shown by Barr and Himwich (1923) by direct determination. Some of their observations covered about the same periods after exercise as in the present experiments, but their samples were not drawn with the same regularity of interval, and do not suffice for the construction of a curve. Insofar as the lowered CO_2 tension is thus determined, it is obvious that the return to normal can occur only as the lactic acid disappears. It must therefore be inferred that the disappearance of the latter occurs at a diminishing rate. That this is indeed the case is shown by the curves of Hill, Long and Lupton (1924a) of the variations of lactic acid in human blood after severe exercise. They all exhibit in their downward course a concavity upwards, and are qualitatively the inverse of the alveolar CO_2 tension curves here presented. It is evident that the disappearance of lactic acid in the blood follows the law of mass action. This would seem to indicate that the disposal of lactic acid in the blood through oxidation, or any other mechanism, is accomplished by usual chemical processes, and not governed by a limited amount of catalyst, as Haldane and Quastel inferred on the basis of the supposed straight line curve of the post-exercise recovery of the alveolar CO_2 tension.

SUMMARY

The curve of CO_2 tension in alveolar air during recovery from a short period of vigorous muscular exercise is concave downward, and is roughly the inverse of the published curves of lactic acid disappearance from the blood, and of the disappearance of the oxygen debt. The form of the curve indicates that the chemical responses giving rise to this behavior of the CO_2 tension are governed by the usual law of mass action and not by limitation of enzyme available.

The author wishes to thank Dr. Joseph Erlanger for suggesting this investigation and for aid during the course of the work.

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SUPRARENAL INSUFFICIENCY

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No attempt is being made in this report to give a complete review of the literature on suprarenals, but merely a few outstanding contributions will be mentioned. The clinical observations of Addison published in 1855 on *The Constitutional and Local Effects of the Disease of the Suprarenal Capsule*, were so complete that little knowledge has been added to the suprarenal problem by the clinician since his time. Pathologists have endeavored to link up the pathological findings with the clinical picture. But here as in the case of diseases of the kidney the autopsy findings are not always what would be expected from the clinical observations. In some cases dying of Addison's disease the autopsy findings show that only one suprarenal has been involved, the other being apparently normal. Experimentally it has been found that following the removal of one suprarenal the animals remain normal. Brown-Sequard (1856) found that if both suprarenals are removed the animals die, provided there is no accessory suprarenal tissue. His animals died twenty-four to forty-eight hours after suprarenalectomy, and the symptoms preceding death in no way resembled Addison's disease. So far it has been impossible to produce Addison's disease in animals.

Each suprarenal consists of a cortical layer derived from the mesoblast and a medullary portion derived from the ectoderm, as an outgrowth of the sympathetic plexus. In certain fishes these two portions exist separately, but in all higher forms, particularly the mammalian vertebrates, the cortex is intimately connected with the medullary portion. Many species of animals especially the rat and the rabbit, have accessory suprarenal tissue, and this accounts for their survival after the removal of both suprarenals. According to Biedl (1913) 20 per cent of rabbits and 50 per cent of rats possess this accessory tissue. It is rarely found in cats or dogs and these animals always die after both suprarenals are removed.

Clinicians for the most part have been more concerned with the medullary portion of the suprarenal, particularly since Oliver and Schaefer (1895) isolated epinephrin from this part of the gland. But the work of Biedl, Vincent (1917), Houssay and Lewis (1923) and others (Stewart, 1921), (Tokumitsu, 1923) has shown that it is the cortex and not the medulla

that is essential to life. Biedl found that in certain fishes in which the medulla and cortex exist separately, the removal of the cortical portion resulted in death. He also found that rabbits and dogs survived the removal of seven-eighths of the total suprarenal tissue, provided that the remaining part was cortical tissue. Vincent found that extirpation of one gland, and the cauterization of the medulla of the other, leaving only cortex, did not result in death.

The suprarenal is supplied by three main arteries which ramify in the capsule, sending in branches through the perpendicular columns of the cortex into the medulla. Drainage of the blood from the suprarenal is by the suprarenal vein which empties into the inferior vena cava. This vein is peculiar in that its tributaries arise at the junction of the cortical and medullary tissue in such a way that the blood coming from the cortex and medulla remains separate till entering these tributaries. The thickness of the cortex is irregular and varies in different species of animals, in different animals of the same species, and in the same animal at different phases in development.

Some observers (Hartman and Hartman, 1923), (Cramer, 1918) found that the cortex contained epinephrin. This was possibly due to the fact that the cortex had not been shaved off for some time after the death of the animal. We found epinephrin present even in the outer layers of the cortex two hours after death when the cortex had not been shaved off from the medulla. It appears therefore that the diffusion of epinephrin from the medulla to the cortex might occur very rapidly after death. To prevent this diffusion, and to ascertain whether the cortex was free from epinephrin, it was decided to shave off the cortex in living animal under anesthesia. Large animals were chosen because the thickness of the cortex made it possible to obtain strips of cortex entirely free of medulla. The Connaught Laboratories provided us with one cow and two horses for this purpose. In only one case was it possible to shave the cortex in situ, but in the other two cases the suprarenals were removed and the cortex shaved off within two minutes of cessation of the circulation in the glands. The cortex shaved off in this way did not contain epinephrin in estimable quantities by the chemical tests (Folin, Cannon and Denis, 1912) and when tested on rabbit intestine a one-in-ninety solution gave no epinephrin reaction although a marked response was obtained with a one-in-five-million solution of the drug.

Having satisfied ourselves that it is the cortex and not the medulla that is essential to the life of an animal, and also that the medullary portion can be removed by cautery without affecting the health of the animal, it was decided to study the effects of suprarenalectomy. Dogs were chosen for this series of experiments because of the ease with which samples of blood can be obtained, and because they seldom, if ever, possess any acces-

sory suprarenal tissue, and invariably die when the suprarenals are removed.

There is a marked individual variation in dogs. In some the suprarenals are removed with comparative ease while in others, owing to anatomical location and the firmness with which they are attached, removal is extremely difficult. We have found the least difficulty with young, fully grown dogs. Soon after commencing this work it was found that the length of time of survival depended largely on the operative technique and as a result the operative procedure received special attention. The operation is carried out as follows. The dog is anaesthetized. After preparation of the skin a three inch incision is made extending from the angle of the last rib distally and slightly ventrally. Dissection is carried around the edge of the lumbar muscle, and the muscle retracted so as to expose the ventral layer of the dorsal fascia. This layer is slit and separated, care being taken to avoid as far as possible damage to the vessels and nerves. The retroperitoneal tissue behind the kidney is thus exposed. On the level with the upper pole of the kidney running outward from the midline over the suprarenal there is invariably to be found a large vein. This vein is the guide to the suprarenal. It is dissected free and clamped and is then followed to the gland. It is again clamped close to the suprarenal and divided. The suprarenal gland is very fragile and damage to it results in hemorrhage which adds to the difficulty of the operation. If the clamp on the vein is placed close to the gland there is no necessity of grasping the gland itself, for this clamp holds the gland firmly enough to allow for the necessary manipulation. The gland is then dissected free. In order to prevent hemorrhage while dissecting, care must be taken to keep close to the capsule. The medial portion of the vein remains finally as a pedicle. This is clamped, ligated, and the suprarenal removed. Any hemorrhage is stopped. The wound is closed layer on layer with catgut. Strict asepsis is observed throughout. It is important that the tissue be handled carefully so that as little damage as possible results from the operation. The wound should not be closed till all hemorrhage has been controlled, leaving the area of operation perfectly dry. In the early operations these essential points were not sufficiently observed and it was found that in cases where slight hemorrhage or oozing was left the dogs died comparatively early. Furthermore, the symptoms which are observed in dogs which die within 50 hours are different from those surviving 200 hours. Many of the symptoms of the former are attributable to shock and hemorrhage, whereas in the longer surviving dogs the true symptoms of suprarenal deficiency may be observed.

Since beginning this work we have removed the suprarenals of fifty-six dogs. Sixteen of these had both suprarenals removed at one operation, and forty had them removed in two stages. The time intervening between

the removal of the two glands varied from ten days to six weeks. Previous workers (Elliott, 1914) have shown the removal of one gland causes the hypertrophy of the remaining gland.

The symptoms which preceded death in dogs surviving less than fifty hours were characterized by:

Rapid pulse	Suppression of urine
Elevated temperature	Salivation
Thirst	Vomiting
Loss of appetite	Diarrhea
Terminal fall in blood sugar	Unconsciousness
Restlessness	Coma and convulsions

Many of these symptoms were found to be due to operative shock and were not observed in longer surviving dogs.

In dogs which survive from one hundred to two hundred hours recovery from anesthesia occurs in a few hours. The dogs then appear normal in regard to disposition, thirst, excretion of urine, bowel movement, blood sugar and general clinical condition until two or three days preceding death. The dogs usually refuse food for the first twenty-four to forty-eight hours following operations, but the appetite returns and remains fairly normal until a day or two before death. The pulse, respiration and temperature usually increase. Healing of the wounds takes place as in normal dogs. A noticeable symptom is the inflammation at the margin of the gums. This first appears as a red band and then becomes blue and at death is dark blue.

The fatal termination in dogs surviving over one hundred hours may begin with suppression of urine, loss of appetite, weakness, especially in the hind legs, drowsiness, pus gathering in the eyes and the pupils becoming dilated. The superficial leg veins collapse and blood samples are obtained with difficulty. The blood becomes thick and dark. The serum does not settle out on standing, and only small quantities are obtained on centrifuging. Finally, within a few hours of death there may be vomiting and diarrhea. The bowel movement at first is normal in color but soon becomes dark brown and at death is reddish brown. The blood sugar and temperature usually fall below normal and the dog loses consciousness. Frequently the respirations become slow, even to 9 or 10 per minute, and are abdominal in type. Immediately preceding death the respirations become more rapid. The heart continues to beat some minutes after the cessation of the respirations.

It may be advisable to emphasize the contrast in the terminal symptoms between short and long surviving dogs. In dogs which survive fifty hours or less there is usually persistent vomiting, restlessness, thirst and diarrhea, whereas in dogs which survive one hundred and fifty to two hundred hours these symptoms may not occur until within four or five hours of death and are usually much less severe.

Various means were tried in the endeavor to modify these symptoms.

1. Repeated doses of epinephrin did not prolong the life of the animal. For example, dog 12 (two-stage removal) received $\frac{1}{4}$ cc. of 1/1000 solution of epinephrin every five hours and survived sixty-one hours.

2. Repeated injections of glucose prolonged life a few hours and kept the blood sugar up till the time of death.

3. Various types of beef cortex extract did not prolong life nor modify the terminal symptoms, but in many cases appeared to shorten the period of survival due probably to their protein content.

4. Extract of dog suprarenal cortex appeared to prolong life in desuprarenalized dogs and also gave marked clinical improvement following its administration. The extract was prepared as follows. The gland was removed, cut lengthwise, and the medulla scraped out. The cortex was macerated with sand, and extracted with distilled water. An example of the result of this treatment is shown by dog 24 (two-stage removal). This dog received freshly prepared dog cortex extract in divided doses every day for a week. It survived one hundred and seventy-five hours and the terminal symptoms appeared very suddenly and were of short duration.

5. Normal dog blood serum injections prolonged life and resulted in improvement in the clinical condition. See dog 117.

6. Repeated saline injections prolonged the life of the dog and resulted in marked clinical improvement as long as the kidney function was not impaired. See dog 117.

Having gained a knowledge of the symptoms of suprarenal insufficiency it was thought that the investigation of the blood chemistry might throw further light on the problem. This work was begun by Doctor Lucas in October, 1924 and is being published with this article. Samples of blood were examined before, and at various intervals after suprarenalectomy. Chemical changes of the blood following the removal of one suprarenal did not differ from the normal.

The outstanding chemical changes of the blood after complete suprarenalectomy may be briefly summarized as follows. The total solids, total nitrogen, urea and non-protein-nitrogen are all increased. The two former increase gradually from the time of operation till death, the two latter increase more rapidly immediately preceding death. In four dogs surviving from 100 to 200 hours the average increase in total blood solids was from 21.12 per cent to 24.09 per cent; in total nitrogen from 3.23 per cent to 3.92 per cent; in urea from 15.77 to 77.73 mgm. per 100 cc. blood; and in non-protein nitrogen from 36.8 to 140.0 mgm. per 100 cc. blood. The blood chloride decreases in the terminal stage. In the earlier experiments it was found that the blood sugar decreased immediately preceding death. The blood calcium and cholesterol increase slightly but the increase can be

accounted for by the thickening of the blood. In dogs which received saline there was no thickening of the blood and the calcium and cholesterol remained practically normal.

To illustrate the effect of hypertonic saline on longevity and blood chemistry, dog 117 is reported in detail.

On May 19, 1925, the right and left suprarenals were removed and six hours later the dog received 50 cc. dog serum intravenously.

On May 20, the dog received 150 cc. serum in three doses. The urea on this day was 25.63 mgm. per 100 cc. blood.

On May 21, the dog received 175 cc. serum in three doses.

On May 22, the dog received 195 cc. serum in three doses, and 150 cc. normal saline. The urea was 10.73 mgm.

On May 23, the blood urea was 13.51 mgm., and the N.P.N. 36.7 mgm. per 100 cc. blood. The dog received 110 cc. serum in two doses.

On May 24, the dog received 100 cc. serum in two doses.

On May 25, the dog received one dose of serum, 50 cc. Urea 20.97 mgm.

On May 26, saline and serum were discontinued, the dog being in excellent condition.

On May 27, blood urea was 26.56 mgm.

On May 28, the dog had been given meat, and two hours later the urea was 52.66 mgm., and the N.P.N. 80.0 mgm.

On May 29, the dog was now receiving all the meat she would eat. The urea was 90.0 mgm. and the N.P.N. 160 mgm. Blood was difficult to obtain and clotted readily.

On May 30, the urea before the dog was fed was 141.2 mgm. An hour and a half after receiving the meat the urea was 144.5 mgm. and the N.P.N. 186.0 mgm. The dog was now given 150 cc. serum and 50 cc. saline. The urea an hour and a half after saline was 102.99 and the N.P.N. 197.52 mgm.

On May 31, the dog had the first loose bowel movement. The urea at 9:00 a.m. was 147.26 and the N.P.N. 199.0 mgm. The dog was given three doses of serum (170 cc.) between 11:00 a.m. and 1:00 p.m. and at 2:30 p.m. the urea was 157.97 and the N.P.N. 226.9 mgm. From 2:30 p.m. to 4:15 p.m. the dog received 130 cc. serum and at 4:15 p.m. the urea was 165.43 mgm. It is to be noted that despite the fact that the dog received 300 cc. serum in five hours the urea rose from 147.26 to 165.43 mgm. One hundred cubic centimeters of 5 per cent saline were given at 4:20 p.m. and the urea followed at 1½-hour intervals for five hours. The results were, 146.79, 132.81 and 128.51 mgm. At 10:45 p.m. the dog was given 100 cc. 5 per cent saline.

On June 1, at 6:45 a.m., the urea had dropped to 89.47 mgm. One hundred cubic centimeters 5 per cent saline were given and in 1½ hours the urea had fallen to 67.0 mgm. and the N.P.N. was 108.18. The dog received 175 cc. 5 per cent saline and at 5:00 p.m. the urea was 41.94 mgm. On June 2 the urea had dropped to 28.36 mgm.

When the urea and N. P. N. were extremely high, following high protein diet, the dog's condition was characteristic of the terminal symptoms of desuprarenalized dogs. However, when the urea and N. P. N. had returned to their normal level the dog appeared bright and active, though showing slight signs of weakness.

Following the administration of saline the dog passed large quantities of urine.

On June 3, the urea was 31.69, N.P.N. 55.55, and blood chlorides 0.429 per cent. Saline administration was discontinued temporarily.

On June 4, the dog had vomited and had a bowel movement. The urea had risen to 61.05 and the N.P.N. to 97.05 mgm.; the blood chlorides had dropped to 0.396 per cent. The dog began to have intermittent spells of excitement and was noticeably weaker with incoördination in the movements of the hind legs. One hundred cubic centimeters 5 per cent saline were given and 1½ hours later the urea was 59.18, the N.P.N. 103.2 mgm. and the blood chlorides had gone up to 0.453 per cent. At 8:20 p.m. the dog had a convulsive seizure and died. At death the urea was 79.69, the N.P.N. 152.85 mgm. and the chlorides 0.453 per cent.

This dog illustrates the effect of hypertonic saline on the urea which fell from 165.43 to 28.36 mgm. in 24 hours; the N.P.N. which fell from 226.9 to 55.55 mgm. The initial fall of N.P.N. is not as rapid as that of urea. Dog serum alone did not result in a fall in urea and N.P.N., and finally when the kidneys failed to excrete the administration of saline did not result in lowering of urea or N.P.N.

It is to be noted that the dog on May 31 had a urea of 165.43 mgm. and an N.P.N. of 226.9 mgm., while at the time of death the urea was only 79.67 and the N.P.N. 152.85 mgm. This would indicate that the retention of ordinary end products of protein metabolism were not responsible for the death of the animal.

The autopsy findings of this dog were characteristic of all long surviving desuprarenalized dogs. There were enlarged lymphatics, spleen and thymus; the stomach contained ulcers, and the mucous membrane of the duodenum and rectum was congested.

In short surviving dogs (24 to 50 hours) the autopsy findings are characterized by extreme congestion of liver, kidney, pancreas, spleen, omentum and intestine. The lymph glands are congested but not markedly enlarged. The urinary bladder is empty and contracted, and the gall bladder is distended. The bowel frequently contains a dark brown semifluid.

In dogs surviving 150 hours and over, the congestion is much less marked or may be absent in the kidney, liver, and spleen; the pancreas usually showing considerable congestion. There is a very marked enlargement of the lymphatic glands throughout the body, abdominal mediastinal, axillary and cervical regions. The thymus is enlarged and thickened. The spleen is enlarged and firm in consistency. The heart and lungs are apparently normal. The outstanding feature in the long surviving dog, in addition to the enlargement of the lymphatics is to be found in the gastrointestinal tract. As mentioned, before the gum margin is extremely congested and bluish in color, and sometimes ulcers appear under the

tongue. The oesophagus is usually normal in appearance. As previously described by Mann (1916) ulcers frequently occur in the stomach. These ulcers, which are found in the pylorus may be small, numerous, punched out and unaccompanied by inflammatory reaction, or there may be areas of extreme submucal ecchymosis. The stomach usually contains a dark brown or greenish bile and blood-containing fluid. The mucous membrane of the duodenum is frequently congested. The small and large intestine do not share in the congestion but contain a dark brown content. The mucous membrane of the rectum is also congested. In long surviving female dogs the mucous membrane of the uterus is usually congested.

On section the lymph glands, particularly those in the retroperitoneal region are brownish in color, whereas those in the neighborhood of the cecum are softer and on section exude a milky fluid containing polymorphonuclear and endothelial leucocytes.

In all cases a careful search was made for suprarenal or accessory suprarenal tissue, but none was found.

We have not observed any change in the pigmentation of the skin.

Blood drawn from the heart at death is thick and dark brown in color. It does not clot normally but remains fluid on standing. Only small quantities of serum can be obtained on centrifuging.

Sections were made of the various tissues of dogs surviving various lengths of time. The principal changes have been found in the liver, lymph glands and kidney.

Sections of the liver show much degeneration in the liver cords varying in degree to actual necrosis of the cells. There is great vascular congestion and hemorrhage which is more marked in areas about the central vein, with pressure changes in the liver cords with fatty degeneration and actual necrosis. In many of the longer lived dogs there is very little liver tissue left. A few scattered cords of the liver are found, usually in the neighborhood of the portal vein. A careful search was made for the centres of hematapoesis described by Wislocki and Crowe (1922) but none were found.

A very constant finding in the lymph glands has been the enlargement with edema, and the tremendous proliferation of plasma cells, endothelial leucocytes, enlarged macrocytes, and occasionally polymorphonuclear leucocytes. There is a great increase in the reticular tissue in many glands. The germinal centres are compressed and almost obliterated. The newly formed sinuses are filled with albuminous fluid and the remainder of the gland is filled with albuminous material and frequently with red blood cells. Large numbers of the plasma cells are usually found. Endothelial leucocytes and other phagocytes are loosely packed together and show active phagocytosis of red and white blood cells. They frequently contain much blood pigment, especially in the dogs which received blood

transfusions. There is a varying degree of stimulation of the reticulo-endothelial cells and the picture varies accordingly. There was no evidence found of regeneration of the red blood cells.

The sections of the kidney showed a general hyperemia with small focal areas of hemorrhage throughout. The glomerular tufts were little changed but there was a general swelling of all the tubules. The epithelial cytoplasm was pale and granular, and the cell margins were ragged and indistinct. The nuclei stained very well. The lumen of the tubules showed the presence of albuminous casts, and red and white blood cells. There was only a slight sign of regeneration in badly damaged tubules and occasionally a very slight scattering of lymphocytes through the interstitial tissue.

Sections of the gastric and intestinal mucosa showed superficial ulceration with edema, congestion and hemorrhage with denudation of the epithelium. There was an inflammatory exudation of endothelial and lymphoid cells in the adjacent areas.

The splenic sinuses were filled with disintegrating red blood cells, phagocytes of different sorts, the Malpighian corpuscles and other histological elements being little altered.

There were no outstanding structural changes or constant pathological condition found in the other tissues examined.

It is thought by many observers that the suprarenals exert an important influence on carbohydrate metabolism. This idea arose from the fact that epinephrin produces an increase in the percentage of sugar in the blood. Furthermore many observers have found that when the suprarenals are removed there is a rapid decline in the blood sugar.

Bornstein and Holm (1923) noted the low blood sugar in dogs dying after double suprarenalectomy, but since in depancreatized dogs the blood sugar remained high they concluded that this was not the cause of death.

To show that the death of the animal was not due to this decline in blood sugar we depancreatized dog 10 (February, 1924) and gave insulin for one week, at the end of which time both suprarenals were removed. The animal survived double suprarenalectomy six hours, the blood sugar rising from 0.224 to 0.434. This experiment was repeated, the dog surviving 30 hours, the blood sugar rising from 0.243 to 0.408.

In our early experiments dog 8 survived 12 hours, the blood sugar dropping from 0.098 to 0.048. This decline could be controlled by the administration of glucose injections. But whereas the life of the animal was slightly prolonged by these injections, death invariably ensued.

In the early experiments it was found that the lowering of the blood sugar did not depend on the absence of suprarenal but was the result of the shock of the operation, or occurred as a terminal symptom. Recently it has been found that in a dog 24 hours after suprarenalectomy the toler-

ance to carbohydrate metabolism was unimpaired as shown by the fact that the blood sugar curve following the intravenous injection of 2 grams carbohydrate per kilo body weight was the same as in a normal dog. It was found that the subcutaneous effect of epinephrin was also followed by a rise in blood sugar (0.063 to 0.136). Four dogs dying within 20 hours after double suprarenalectomy showed less than 0.5 per cent glycogen in the liver; whereas one dog surviving 217 hours showed 1.2 per cent glycogen, and dog 12 which received glucose injections and survived 61 hours had 2.04 per cent glycogen in the liver despite the terminal symptoms. From these findings it may be concluded that the suprarenal cortex does not play a direct rôle in carbohydrate metabolism. This belief is also held by Stewart (1924).

Professor Henderson pointed out that the blood picture and clinical findings of desuprarenalized dogs closely resembled the findings in delayed shock. On this suggestion a comparison was made of the toxicity of varying doses of histamine before and after suprarenalectomy.

Dale (1921) found that the blood pressure lowering effect of histamine was ten times greater in desuprarenalized than in normal cats.

Lewis (1923) had found that, "double adrenalectomy in albino rats increased their sensitiveness to morphine, codeine, papaverine, curare cobra venom, veratrine, digitoxin, adrenalin and diphtheria toxin. With picrotoxine and strychnine no difference was observed."

In our experiments the histamine was given subcutaneously as a 1 per cent solution. Dog 77 received 20.8 mgm. histamine per kilo body weight on January 18. This dose produced vomiting, diarrhea, labored breathing and an expiratory grunt. The dog had completely recovered in six hours. On January 19, the right suprarenal was removed and on February 2, the left suprarenal was removed. Eighteen hours after the removal of the second suprarenal the dog appeared in excellent condition and the same dose of histamine was given. The dog died in $1\frac{1}{2}$ hours after the injection.

On February 7, dog 82 completely recovered in two hours after 10 mgm. histamine per kilo body weight. On February 9, the left suprarenal was removed and on February 26, the same dose of histamine was given. The dog recovered in six hours. On February 27, the right suprarenal was removed. Twenty-four hours later the dog was in excellent condition and the same dose of histamine was again given. The dog became unconscious within five minutes and died in half an hour.

On February 14, the right suprarenal was removed from dog 85, and on March 5 the left suprarenal was removed. On March 7, (42 hours after the removal of the second suprarenal) 2 mgm. of histamine per kilo body weight produced death in $3\frac{3}{4}$ hours.

Dog 116 had the right suprarenal removed on May 16, and the left on June 18. Seventy-four hours after the removal of the second suprarenal

the dog was in good condition and 1 mgm. of histamine per kilo body weight was given. In half an hour the dog was semi-conscious, and died an hour after the injection.

That this toxicity cannot be counteracted by epinephrin is shown by dog 88. Twenty-four hours after the removal of the second suprarenal this dog received 10 mgm. histamine per kilo body weight and at the same time $\frac{1}{2}$ cc. epinephrin subcutaneously. It received 1 cc. 1/1000 solution epinephrin every ten minutes for half an hour, at the end of which time the animal died.

Similar experiments were carried out with intravenous injections of Witte's peptone. Dog 90 received 16 cc. of a 4 per cent solution (116 mgm. per kilo) eighteen hours after the removal of the second suprarenal and died in thirty minutes, whereas a normal dog, 106, received 75 cc. of the same solution (260 mgm. per kilo) and fully recovered in five hours. The histamine content of the peptone was negligible, being estimated as 0.003 per cent.

From these and other experiments it appears that histamine is not equally toxic to all normal dogs, but it is about thirty times more toxic after double suprarenalectomy.

From the above findings it seemed reasonable to believe that in the normal animal the suprarenal enables the body to handle poisons which in the absence of the suprarenals accumulate producing death. Many subsidiary findings tend to substantiate this view. For instance it was found that intercurrent infections such as distemper, infected wounds, tissue damage and hemorrhage at the time of operation, all tend to shorten the time of survival. The period of survival in dogs which received extracts of foreign protein was shortened. Desuprarenalized dogs appeared to be hypersensitive to foreign protein.

Since the lymphatic glands in all areas of the body were enlarged it was thought that they might contain a toxic substance. The lymph glands of dog 63, which survived 93 hours after the removal of the second suprarenal were extracted with 150 cc. normal saline and 30 cc. were injected intravenously into dog 62. This dog had had $1\frac{1}{2}$ suprarenals removed about a month previously but up till this time had appeared quite normal. When 30 cc. of the extract had been given the dog had a reaction characterized by stiffening of all four legs, defecation, urination and cessation of respiration. Artificial respiration brought about recovery. Five minutes later the remaining 120 cc. of extract were injected slowly; the dog became unconscious and had a bowel movement. During the next nine hours there was frequent diarrhoea and vomiting, and the dog remained unconscious. The dog did not regain full consciousness; it had intermittent howling and was hypersensitive in the hind legs. The dog appeared to be suffering, so was chloroformed. Post-mortem examination did not differ markedly

from early suprarenal death, there being marked congestion of the liver, kidneys, spleen and pancreas. There was an intense hemorrhagic appearance in the neighborhood of the half suprarenal. On histological examination the suprarenal tissue was found to be extremely congested with areas of round cell infiltration and transudation of red blood cells into the tissue. Evidence of degeneration of all three layers of cortex was less marked in the glomerular layer. The fascicular and reticular layers showed fatty degeneration.

It would seem from this experiment that the extract of the enlarged lymph glands contains sufficient toxic substance to overwhelm the small amount of suprarenal tissue present. However on repeating this experiment there was but a slight reaction following the intravenous injection into a normal dog of 200 cc. of extract made from 21 grams of lymph gland tissue removed from dog 74 which survived 120 hours. Boiled extract of lymph glands from dog 65, which survived 217 hours, was non-toxic to a normal dog.

Langlois (1892) found that the injection of blood from a dog dying after removal of both suprarenals was non-toxic when injected into a normal dog or a dog having one-eleventh of the suprarenal tissue left after a two-stage double removal. We found that 400 cc. blood from a dog dying 24 hours after double suprarenalectomy had no effect when injected intravenously into a normal dog. Recently we have found that 80 cc. serum from a dog dying 16 days after double suprarenalectomy was sufficient to produce diarrhoea, vomiting and general malaise lasting 6 to 8 hours in a normal dog. It would seem therefore that the dog which survived 24 hours died from operative shock rather than from the accumulation of toxic substances which give rise to true suprarenal death.

As mentioned previously, the frequent administration of saline prolonged the life of the desuprarenalized animal. The fact that frequent administrations of saline, which promotes elimination by the kidney, prolonged the life of the desuprarenalized animal is another indication that there is an accumulation of a toxic substance following suprarenalectomy. Another fact which points to the same idea is that two dogs, 67 and 69, which had one suprarenal removed and the medulla of the other cauterized, appeared in good condition for three months, at the end of which time they were put on a high protein diet. Within a week after beginning this diet both the dogs showed typical symptoms of suprarenalectomy, and death occurred in three to four days.

SUMMARY

Up to the present we have studied the symptoms, blood chemistry and histological pathology of 16 dogs which had both suprarenals removed at one operation and of 37 dogs in which an interval of 10 days to 6 weeks

intervened between the removal of the first and second gland. Of the 16 dogs in which double suprarenalectomy was performed all died in from 8 to 58 hours. The average length of survival was 26.8 hours, with the exception of dog 117, previously mentioned, which survived 388 hours. Of the 37 dogs in which suprarenalectomy was performed in two stages, twelve dogs survived less than 50 hours, ten survived 50 to 100 hours, ten survived 100 to 150 hours, three survived 150 to 200 hours and two survived from 200 to 238 hours. With regard to length of survival these results are slightly better than those obtained by Biedl (1913) or by Strehl and Weiss (1901), but are not as good as those reported by Stewart and Rogoff (1925) who had dogs survive 1 to 16 days without treatment, and 6 to 34 days with saline-dextrose treatment.

The tendency for an increase in the urea and non-protein nitrogen, and the decrease in the percentage of chlorides in the blood are the outstanding chemical changes. The increase in urea and non-protein nitrogen seem to depend in some way on the ability of the kidney to excrete, since the administration of saline only reduces them when accompanied by diuresis. The percentage of chloride in the blood can be maintained at its normal level by saline administration regardless whether the kidney is excreting or not. Up to the present we have been unable to prevent death in desuprarenalized dogs even when the urea, non-protein nitrogen, and chloride have been maintained at an almost normal level.

The outstanding post-mortem findings of enlarged lymph glands, degeneration of the liver cords, and nephrosis of the kidney strongly indicate the accumulation of a toxic substance. Further evidence to support this fact is that the blood of dogs which died a true desuprarenal death is toxic. A protein diet with its consequent accumulation of the protein-split products shortens the duration of survival and hastens death in partially desuprarenalized dogs. The fact that histamine is thirty times more toxic after suprarenalectomy suggests that the poison is of a protein-like nature.

The difficulties encountered at the operation, combined with the possibilities of post-operative complications, introduce perplexing factors which must be eliminated before a comprehensive study of suprarenal insufficiency can be made. The problem of why animals die after suprarenalectomy is far from being solved.

We wish to express our thanks to Prof. V. E. Henderson for helpful suggestions and the facilities offered in his department.

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BLOOD AND URINE FINDINGS IN DESUPRARENALIZED DOGS

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The chemical analyses reported in this paper were made in this laboratory in conjunction with Dr. F. G. Banting and Miss S. Gairns and were carried out as part of the study (Banting and Gairns, 1926) undertaken by Professor Banting to whom the author's thanks are due for material and constant supervision. During their research on suprarenal insufficiency they were unable to find in the literature sufficient chemical data to give them any clue as to what changes were taking place in the blood of dogs after double suprarenalectomy. Therefore in October of 1924 a chemical study of the blood and urine of the dogs before and after operation was begun in an effort to determine the effect of the removal of the adrenals from the body.

The methods employed were those generally in use and are as follows. For blood chlorides, Whitehorn (1921); non-protein nitrogen and creatinine, Folin and Wu (1919); uric acid, Benedict (1922); cholesterol, Bloor (1916); urea, Van Slyke and Cullen (1914); calcium, Kramer and Tisdall (1921); phosphorus, Tisdall (1922). Total nitrogen was estimated on 2.0 cc. sample of blood by the Kjeldahl method while total solids were obtained by drying a weighed quantity of blood, about 2 grams, to a constant weight at 100°C. In urine analysis the method of Van Slyke and Cullen was used for urea and ammonia while that of Volhard-Arnold (Hawk, 1923) was employed for chlorides. Total nitrogen was estimated on 5.0 cc. of the urine by the Kjeldahl procedure.

Blood for analysis was obtained usually from a leg vein but when the blood became more concentrated after removal of the suprarenals and was very difficult to draw from a vein occasionally a sample was taken from the heart; this latter method was avoided as much as possible. Whenever it was essential the sample was collected under oil. Urine samples were collected from the metabolism cages and were preserved under toluene for analysis.

The changes in the blood and urine of dogs after the removal of one suprarenal gland are practically negligible (table 1) and in this work very often the blood from a dog from which one gland was removed was called normal. In the majority of cases the glands were removed in two-stage

operations; for details see publication of Banting and Gairns (1926). The blood and urine were analysed three or four days previous to the removal of the second gland and following that until the dog died. The characteristic changes taking place in dogs after double suprarenalectomy are indicated below.

Total solids. The blood after double suprarenalectomy begins to concentrate at varying stages after the operation; it becomes darker in color and is very difficult to draw from the veins in the leg as the vein collapses. This concentration is quite noticeable when the blood is centrifuged for the amount of corpuscles may increase from about 50 to 60 per cent while the plasma correspondingly decreases. Another noticeable change takes place in the blood when dried for 24 hours at 100°C. Normal blood or blood after removal of one gland forms a thin, hard, elastic button in the weighing bottle, whereas the blood of the dog after removal of both glands becomes very brittle on drying and breaks up into many pieces. This brittleness becomes more pronounced as death approaches.

Total nitrogen. The increase of total nitrogen in the blood is due no doubt to the increased concentration of the proteins. During the precipitation of proteins from samples of serum with trichloracetic acid in the estimation of calcium considerable difference in the amount of precipitate was noticed. On centrifuging the protein from normal serum about 0.6 cc. of precipitate was obtained whereas as much as 0.9 cc. of precipitate was observed from 1.0 cc. of serum of dog dying from removal of both glands. Table 3 gives some determinations (grams of total nitrogen per 100 cc. blood) typical in dogs dying from double suprarenalectomy.

Urea and non-protein nitrogen. The urea and non-protein nitrogen in the blood increase after double suprarenalectomy. The accumulation in dogs which live about 48 hours is quite rapid and begins soon after the operation (table 9); whereas in dogs of long survival the increase is more of a terminal stage phenomenon (tables 10 and 11). The figures obtained from these dogs are not as high as one finds in dog's blood after ligaturing the ureters, and it is entirely unlikely that death is due to the accumulation of these constituents. Table 4 gives in milligram per 100 cc. of blood the typical urea and non-protein nitrogen accumulation in a number of dogs dying from double suprarenalectomy and one from ligaturing of the ureters.

Uric acid and creatinine. Several estimations of uric acid were made to be certain no large increase was taking place. The creatinine was found to increase but slightly and very few determinations were made. The results of the analyses are given in table 5.

Cholesterol. Owing to its cholesterol content the suprarenal cortex has been supposed to control the cholesterol metabolism in the organs of the body. It has been pointed out by Baumann and Holly (1923) that the

TABLE 1

Results of analysis on the blood of dog 51 before and after removal of right suprarenal on November 17, 1924

DATE	TOTAL NITROGEN	TOTAL SOLIDS	CHLORIDES	UREA PER 100 CC. BLOOD	N.P.N. PER 100 CC. BLOOD	BLOOD SUGAR PER 100 CC. BLOOD
	per cent	*per cent*	*per cent*	*mgm.*	*mgm.*	*mgm.*
November 13	3.11	21.37	0.421	3.80	34.4	0.098
November 18	2.98	20.31	0.445	6.40	29.7	0.088

TABLE 2

Typical increase in total solids (grams per 100 grams of blood) in the blood of dogs following double suprarenalectomy

	DOG 45	DOG 49	DOG 72	DOG 63	DOG 74	DOG 54	DOG 48
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Normal	19.07	19.14	21.61	20.24	21.27	21.73	21.27
After double suprarenalectomy at death	20.17	23.58	24.20	23.51	25.08	23.33	24.47
Hours of survival	37.5	42.5	49.0	93.0	127.0	145.0	199.0

TABLE 3

	DOG 45	DOG 49	DOG 72	DOG 63	DOG 74	DOG 54	DOG 48
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Normal	2.79	2.79	3.22	3.20	3.26	3.30	3.15
After double suprarenalectomy	3.09	3.71	3.86	3.49	3.94	3.54	3.82
Hours of survival	37.5	42.5	49.0	93.0	127.0	145.0	199.0

TABLE 4

DOG NUMBER	UREA PER 100 CC. BLOOD			N.P.N. PER 100 CC. BLOOD			HOURS SURVIVED
	Normal	Death	Ureters ligated	Normal	Death	Ureters ligated	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
45	4.20	42.5		25.0	94.7		37.5
49	12.0	58.4		33.4	131.0		42.5
63	10.47	30.0		29.1	124.5		93.0
74	8.39	89.9		25.1	133.6		127.0
54	30.10	124.0		55.2	212.0		145.0
48	14.10	66.2		37.4	90.0		199.0
56	13.2		197	36.9		300	Chloroformed after 73 hours

TABLE 5

DOG NUMBER	URIC ACID PER 100 CC. BLOOD		CREATININE PER 100 CC. BLOOD		HOURS SURVIVED
	Normal	Desuprarenalized	Normal	Desuprarenalized	
	mgm.	*mgm.*	*mgm.*	*mgm.*	
36	0.69	0.80	1.35	1.60	50.5
45	0.80	0.71			37.5
49			1.42	1.85	42.5

TABLE 6

	DOG 45		DOG 36		DOG 74		DOG 65	
	Cholesterol	Total solids	Cholesterol	Total solids	Cholesterol	Total solids	Cholesterol	Total solids
	mgm.	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*	*mgm.*	*per cent*
Normal	226	19.07	214	19.63	177*	21.27		18.09
At death following double suprarenalectomy	268	20.17	230	20.58	206	25.08	182	19.15
Hours of survival	37.5		50.5		127		217	

No. 36, 45, whole blood
No. 65, 74, serum
* Sixty hours before death.

TABLE 6A

	TOTAL CHOLESTEROL PER 100 CC. PLASMA	TOTAL ESTER PER 100 CC. PLASMA	PER CENT OF ESTER PRESENT IN PLASMA CHOLESTEROL
	mgm.	*mgm.*	
Dog 144:			
Normal	182	79	43.4
After 140 hours	278	123	43.4
Death (after 158 hours)	313	167	53.2
Dog 149			
Normal	208	97	41.7
48 hours	244	114	47.0
56 hours, death	245	121	49.5

Suprarenals

DOG NUMBER	GLAND NUMBER	WEIGHT OF GLAND	PER CENT CHOLESTEROL	PER CENT CHOLESTEROL ESTER	PER CENT ESTER OF TOTAL	GLAND NO. 2 REMOVED AFTER
		gram				*days*
147	1	0.590	4.1	2.2	54.3	32
	2	0.740	3.4	2.0	55.4	
148	1	0.610	8.2	5.9	72.0	
149	1	0.615	6.7	4.7	71.0	38
	2	0.610	8.1	5.2	64.8	

cortex is too small to be a storehouse for cholesterol and they found in rabbits after double suprarenalectomy but slight changes in this constituent. The analyses made in this laboratory on whole blood, serum and plasma confirm the observations of the above mentioned workers; the results of analyses made are indicated in table 6, giving the cholesterol and total solids per 100 cc. of whole blood and serum.

These changes in total cholesterol may be due to the increased concentration in the blood; at all events the removal of the suprarenals has made no striking changes in the cholesterol content of the blood stream. There is a possibility that the removal of the glands may disturb the ratio of cholesterol esters to the free cholesterol in such a degree as to bring about serious changes in the blood. This ratio in blood and in suprarenal glands was measured; from the results reported in tables 6 and 6A on cholesterol it is apparent that the suprarenal gland plays a negligible part in cholesterol metabolism.

TABLE 7

Chlorides (as NaCl) per 100 cc. blood

	DOG 52	DOG 49	DOG 63	DOG 74	DOG 48
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Normal	454	400	519	495	499
At death	376	384	396	397	392
Hours of survival	36	42.5	93	127	199

Blood chlorides. The percentage of chlorides in the blood of dogs shows a decided decrease; in table 7 is given the results of a number of analyses of blood typical of dogs dying from double suprarenalectomy.

Calcium and phosphorus. The analyses made show that in the serum there is a small increase of calcium which may be easily accounted for by the increase in concentration of the blood. The phosphorus content of blood is distributed between the serum and corpuscles and varies with the food. No attempt was made to regulate the food given the dogs for very often severe fits of vomiting caused all the food to be lost. In estimating the phosphorus the only precaution taken was to have a minimum amount of hemolysis and to separate the serum from the corpuscles as quickly as possible. The results of analyses made for these constituents are given in table 8.

Urinary constituents. A check was kept upon urinary nitrogen and chlorides. In some of the first analyses ammonia, urea and creatinine were estimated but as the amount of work increased it was considered advisable to estimate only the two mentioned above. The nitrogen output varied considerably but was about 50 per cent of the normal amount; the chlorides varied widely and very little information could be gained from

TABLE 8

Ca and P per 100 cc. serum

	DOG 72		DOG 74		DOG 65	
	Ca	P	Ca	P	Ca	P
	mgm.		*mgm.*	*mgm.*	*mgm.*	*mgm.*
Normal	10.87		10.70	6.4	10.30	4.7
Death	11.30		12.80	26.1	10.39	8.2
Hours of survival	49		127		217	

TABLE 9

DATE	TOTAL NITROGEN	TOTAL SOLIDS	UREA PER 100 CC. BLOOD	N.P.N. PER 100 CC. BLOOD	CHLORIDES PER 100 CC. BLOOD	CREATININE PER 100 CC. BLOOD
	per cent	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
November 6*	2.79	19.14	12.0	33.4	400	1.48
November 15	3.03	20.72	20.6	53.4	425	1.54
November 16	3.71	23.58	58.4	131.0	384	1.80

* Normal blood.

TABLE 10

DATE	TOTAL NITROGEN	TOTAL SOLIDS	UREA	N.P.N.	CHLORIDES
	per cent	*per cent*	*mgm.*	*mgm.*	*mgm.*
November 6*	3.15	21.27	14.1	34.8	447
December 10†	3.16	20.94	9.5	31.6	429
December 11	3.37	21.64	10.8	34.7	
December 15	3.61	23.53	14.6	44.4	396
December 17	3.82	24.47	66.1	90.0	392

* Normal blood.

† December 9, second suprarenal removed.

Urine analysis

DATE	TOTAL NITROGEN	CHLORIDES	VOLUME	TOTAL GRAMS NITROGEN	TOTAL GRAMS CHLORIDES
	per cent	*per cent*	*cc.*		
November 7*	3.27	0.435	290	9.47	1.260
December 11	2.36	0.580	168	3.96	0.974
December 13	0.51	0.085	105	0.54	0.089
December 14	2.63	0.320	125	3.28	0.400
December 15	1.64	0.355	230	3.77	0.820
December 16	1.22	0.555	360	4.39	2.000
December 17	0.59	0.580	85	0.50	0.493

*** Seventy-two-hour sample of urine.**

Normal output per 24 hours: Total nitrogen, 3.16 grams; chlorides, 0.42 gram.

Output following double suprarenalectomy per 24 hours: Total nitrogen, 1.98 grams; chlorides, 0.575 gram.

the figures obtained other than that the kidneys were able to excrete chlorides till the last, despite the kidney damage. Tables 10 and 12 give a fair idea of the urinary figures obtained in the analyses.

Survival and accumulation of blood constituents. In this laboratory dogs have survived double suprarenalectomy from 37.5 to 217 hours, during the course of these analyses. In dogs whose death occurred in about 48 hours the concentration of the blood constituents indicated in the previous pages begins almost with the operation and accumulates rapidly until the end. The analysis of the blood of dog 49 given in table 9 clearly indicates the rapidity of the accumulation.

Dog 48, a fat female, survived the operation 199 hours and in table 10 the results of the blood and urine analyses are indicated. The accumulation of blood constituents was much slower than in dog 49 and appears as a terminal stage phenomenon.

The experiment on dog 48 suggested that the long survival of this dog may have been due to a lower rate of production of toxic substances owing to a decreased metabolism. To substantiate this theory the thyroid glands were removed together with the suprarenals from a small thin dog. Incidentally this dog, 65, survived the operation 217 hours and the analyses of the blood, table 11, indicate much the same result as was found in the case of dog 48. Unfortunately all the urine excreted by this dog was not obtained for analysis.

The blood of dog 65 differed from that of dog 48 chiefly as follows: The total nitrogen and total solids did not accumulate to the same extent and the serum and plasma were obtained quite easily until death. Generally speaking it was found very difficult to obtain serum and plasma from dogs about 48 hours before death from double suprarenalectomy.

It was thought that death following double suprarenalectomy resulted from an accumulation of a toxic substance of protein nature in the body. In order to decrease the amount of toxic substances that might be absorbed from the bowel it was decided to change the intestinal flora of a dog by feeding it bread and acidophilus milk (obtained in the Connaught Laboratories). After dog 74 had received this diet for about a month, a two-stage operation was performed and analyses made on the blood and urine. The results of the analyses as given in table 12 indicate that death occurred practically in the same manner as in the other dogs.

After these analyses were made Dr. V. E. Henderson pointed out the similarity to histamine shock and in his paper Doctor Banting reports his investigation on this subject. Since the livers of the dogs dying from double suprarenalectomy are usually congested and since the liver plays such an important rôle in the problem of metabolism it was considered advisable to search this organ chemically for histamine. Recent workers in this field, Koessler and Hanke (1919), have developed a chemical method

TABLE 11

DATE	TOTAL NITROGEN	TOTAL SOLIDS	UREA	N.P.N.	CHLORIDES	CALCIUM	PHOSPHORUS
	per cent	per cent	mgm.	mgm.	mgm.	mgm.	mgm.
January 21	2.65	18.09	21.3	44.88	561		
January 28						10.30	4.7
January 30	2.57	17 67	18.17	40.20	511	10.00	6.3
February 4			64.77				
February 5			72.23				6.2
February 6	2.97	19.15	89.19	131.40	462	10.40	8.2

TABLE 12

DATE	TOTAL NITROGEN	TOTAL SOLIDS	UREA PER 100 CC. BLOOD	N.P.N. PER 100 CC. BLOOD	CHLORIDES PER 100 CC. BLOOD	Ca PER 100 CC. SERUM	P PER 100 CC. SERUM	CHOLESTEROL PER 100 CC. SERUM
	per cent	per cent	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
January 17*	3.26	21.27	8.39	25.1	495			
January 31	2 98	19.94	7.46	28.9	502			
February 2						10.7	6.4	
February 4	3.61	23.27	18.06	38.2	445			
February 5						11.6	8.2	177
February 7	3.94	25.08	89.94	133.6	379	12.8	26.1†	206

* Normal blood. January 31, blood 12 days after right suprarenal removed. Left suprarenal removed February 2.

† This figure is undoubtedly high because the serum remained in contact with the corpuscles about an hour or more before centrifuging.

Urine analysis

DATE	TOTAL NITROGEN	CHLORIDES	VOLUME	TOTAL GRAMS NITROGEN	TOTAL GRAMS CHLORIDES
	per cent	per cent	cc.		
January 31	1.52	1.18	380	5.78	4.48
February 1	0.85	0.80	665	5.65	5.32
February 2	0.64	0.40	760	4.86	3.04
February 3	1.37	0.84	180	2.47	1.51
February 4	1.20	0.67	375	4.50	2.51
February 5	1.30	0.80	440	5.72	3.52
February 6	1.71	0.62	160	2.74	0.992
February 7	1.77	0.58	290	5.13	1.68
February 7*	1.59	0.50	90	1.43	0.450

* At death.

Normal excretion per 24 hours: Total nitrogen, 5.34 grams; chlorides, 4,26 grams.

Excretion after removal of suprarenals per 24 hours: Total nitrogen, 2.42 grams; chlorides, 1.19 grams.

for the quantitative estimation of histamine in tissue and find histamine a normal constituent of dog liver. They report that guinea pig liver normally is free from histamine but when histamine is injected intravenously or is fed, measurable amounts are found in the liver.

The method employed is that described by Koessler and Hanke with one exception. The third amyl alcohol extraction was made after the silver-baryta precipitation instead of before it because there were less solids and smaller chance of getting interfering substances.

The first series of analyses was made on six livers and the purification, etc., taken to the second last stage. At this stage a quantitative estimation can be made. A good match with the congo red-methyl orange standard was not obtained in each case but table 13 gives the results obtained.

TABLE 13

DOG NUMBER	HOURS SURVIVAL	WEIGHT OF LIVER	HISTAMINE DICHLORIDE		MATCHING IN THE COLORIMETER
			In whole liver	In 40 grams liver	
		grams	*mgm.*	*mgm.*	
1	Normal	393	14	1.40	Too yellow
2	Normal	210	12.5	2.40	Too yellow
3	Normal	330	10	1.20	Too yellow
After double suprarenalectomy					
67	*	309	15	1.94	Too yellow
91	108	360	28	3.11	Fair
106	238	330	61.6	7.70	Almost perfect

* Dog 67 had one suprarenal removed and the medulla in the other cauterized. It died 105 days after this operation.

The second series was carried out to the final stage of purification and chemical analyses were made. Great difficulty was experienced in obtaining a good match in the colorimeter due to a yellowish interfering substance.

When the chemical analyses were completed biological tests were made by Prof. V. E. Henderson on the solutions. The effects of these on guinea pig gut and uterus were compared with a histamine solution and in each case a positive histamine action was obtained. Further tests were made by injecting some of each solution intravenously into a rabbit; some solutions were injected into a dog. In each instance the drop in blood pressure characteristic of histamine was noted and corresponded closely to that obtained when an equivalent amount of histamine was injected.

Discussion. The most noticeable change following double suprarenalectomy is the decided increase in concentration of the blood indicated by the increase in total solids, total nitrogen and the ratio of corpuscles to plasma. The accumulation of a toxic histamine-like substance due to an

increased metabolism or to faulty excretion would alter the permeability of the capillaries and bring about such a concentration (Dale and Laidlaw, 1911). This conception is substantiated by the results obtained from dog 65 where the thyroids were removed. The blood did not become so concentrated and plasma and serum were obtained easily until death. Furthermore, the increase in total nitrogen and total solids was not so great as in many other instances. It does not necessarily follow that death in this latter case is not due to the accumulation of toxic substances for with the removal of both suprarenals and thyroids the resistance of the body may be very much lower and a much smaller amount of toxic material will cause death. In the preceding paper by Banting and Gairns (1926)

TABLE 14

DOG NUMBER	HOURS SURVIVAL	WEIGHT OF LIVER	HISTAMINE DICHLORIDE		MATCHING
			In whole liver	In 40 grams liver	
		grams	*mgm.*	*mgm.*	
4	Normal	272	4.8	0.60	Too yellow
5	Normal	435	6.5	0.60	Too yellow
Ureters ligatured, chloroformed after 73 hours					
126	Ureters ligatured	323	4 8	0.50	Too yellow
After double suprarenalectomy					
127	126	420	8.4	0.80	Too yellow
122	147	402	7.5	0.75	Too yellow
123	198	475	7.8	0.65	Too yellow
117	388	382	10.1	1.06	Too yellow

Numbers 4, 5, 126, 123, quite yellow and match perfectly with each other. Numbers 117, 122, 127, had more orange and matched perfectly with each other but not with standard or with 4 or 5.

attention is drawn to the increased susceptibility to histamine intoxication after removal of the suprarenals.

The accumulation of urea and non-protein nitrogen while quite high is more of a terminal stage phenomenon and may be due to the kidney damage disclosed at autopsy. From the urine analysis it is very evident that the ability to excrete salts and some nitrogenous products is retained to the last stage and ceases about 24 hours before death: it is about this time that there is a rapid increase in these nitrogenous bodies but the amounts of those found are not sufficient to cause death.

The fluctuation in the inorganic constituents is such as might be expected from changes in blood concentration. The small increase in calcium is accounted for by this increase in concentration. There is a phosphate

increase but as the phosphorus content of the plasma varies with the food or any small laking of corpuscles or diffusion from the corpuscles (which have a higher content than the plasma) the amount of increase is of little significance; moreover the blood has become more concentrated. The diminution in blood chlorides is quite in order for with increase in calcium and phosphorus one would expect less chloride. Apparently the disturbance in metabolism of inorganic constituents in no way accounts for death after the operation.

The influence of the adrenals on cholesterol metabolism has been the subject of considerable controversy. As indicated previously, Baumann and Holly have pointed out from their analyses that the glands are too small to serve as storehouses for cholesterol. It is also quite apparent that with the removal of the adrenals there is no loss of cholesterol from the blood but on the contrary there is a gain which is due to the increase of concentration in the blood. From the analyses here reported it is evident that there is but little disturbance in the ratio of free cholesterol to cholesterol esters. The only conclusion that can be drawn is that the adrenals have practically no function in cholesterol metabolism.

Regarding the presence or accumulation of histamine in the dogs dying from double suprarenalectomy no definite conclusions can be drawn. It is quite evident that histamine or histamine-like substances are present in the normal dog liver and the analyses reported seem to favor an accumulation of a histamine-like substance in the livers of these dogs. In connection with this it must be remembered that the sensitivity of animals to histamine intoxication varies widely and in some cases a very small dose will kill while in others a very large amount may be present without exhibiting very serious symptoms (Tisdall, 1926).

SUMMARY

From this study of dogs before and after double suprarenalectomy the following observations have been made.

1. The blood becomes more concentrated and this leads to an increase of total nitrogen and total solids. This increase in concentration begins soon after the operation; it is very rapid in dogs of short survival but is more gradual in long survival.

2. The non-protein nitrogen (urea, etc.) increases up to three or four times the normal amount and is a terminal stage phenomenon.

3. The variation in the inorganic constituents is what one would expect as a result of the increase in concentration of the blood.

4. The removal of the adrenals has no appreciable effect on the cholesterol content of the blood stream nor the ratio of cholesterol ester to free cholesterol.

5. Histamine is a normal constituent of dog's liver and increases slightly after operation.

6. Urine findings indicate that the dogs are able to excrete nitrogenous and inorganic waste until the last stage before death.

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ON SOME ANATOMICAL CHANGES WHICH FOLLOW REMOVAL OF THE PINEAL BODY FROM BOTH SEXES OF THE IMMATURE ALBINO RAT

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The removal of the pineal body was made at 20 days of age and the examination at 85 days. The operated rats were therefore without their pineal body for 65 days during the period of rapid growth which precedes and includes puberty.

The relation of my own results to those previously reported by myself and others will be considered later, in the discussion.

Material. Albino rats from the colony at the Wistar Institute were used. They were taken from a well-bred and thoroughly established strain and could be depended on to show only moderate variability during growth under standard conditions.

The litters used contained four or more individuals and gave at least two of each sex. The operated animals of both sexes and the corresponding controls were thus from the same litter.

At the age of operation—always 20 days—both the controls and those to be operated were found to be somewhat above the table values (Donaldson, 1924, table 144) for body weight on body length and decidedly above the table values (loc. cit. table 157) for body weight on age. They were, therefore, probably a trifle fat and definitely well-grown. At 20 days the lot to be operated exceeded the controls by 3 to 4 per cent in body weight.

When the rats were killed at 85 days of age, the male controls when compared with the table values (loc. cit) were found to be still heavy for their body length, but were slightly (4 per cent) light for their age. On the other hand, the female controls were not only heavy for their length but also slightly heavy (2 per cent) for their age. We conclude from these relations that the control rats grew in such a manner that they may be fairly used as a basis for the necessary comparisons.

Four groups of rats were studied:

1. Those completely pinealectomized: hereafter designated "tests."
2. Those partially pinealectomized: hereafter designated "incomplete."
3. The operated controls.
4. The normal controls: hereafter designated as "controls."

For the discussion which follows, the rats given in table 1 were used.

In this table the operated controls are not entered since the data show that carrying the operation as far as opening the skull—but leaving the meninges intact—does not modify the growth up to 85 days. The first part of the operation is therefore not significant for the subsequent growth of the rat.

The operation. The usual aseptic precautions were observed and ether was used as the anesthetic. Small instruments were employed including a slender straight pair of forceps, finely toothed. These were used to pluck out the pineal body.

Procedure. The hair was clipped from the head and the skin washed with iodine alcohol. An incision of about 1 cm. was made in the skin over the parietal bone just to one side of the midline. With curved scissors an opening was made in the bone and enlarged so as to expose the posterior portion of the cerebral hemisphere. The borders of the opening were kept away from the sagittal and tansverse sutures so as to avoid bleeding from the corresponding sinuses. Such bleeding as occurred was controlled with pellets of cotton. A cut was made in the dura sufficient to allow the insertion of the forceps and these were passed under the dura in a medial direction along the posterior edge of the opening in the skull. Pressing the points of the forceps gently downward the pineal body was firmly caught and plucked out entire. This result can be verified by inspection of the tissue removed.

TABLE 1

Rats here used

SEX	GROUP	NUMBER
Male	Tests	11
Male	Controls	6*
Male	Incomplete	5
Male	Controls	3*
Female	Tests	10
Female	Controls	6*
Female	Incomplete	4
Female	Controls	3*

21 Test
18 Cont.
9 incomplete

* Where there were several tests from the same litter, one control only was used. The numbers in the table are for the controls actually examined.

In no case was the pineal body exposed before it was removed. Sometimes the pineal body is crushed so that a portion of it is left behind. Under these conditions a second attempt at removal was, in some cases, made, but this is undesirable. The bleeding which follows removal is easily controlled. Without replacing the bone the wound was closed by suture of the skin and dressed with collodion. No suppuration occurred.

The operated rats were kept warm and given a liberal supply of milk. After 24 hours they usually became active and for the rest of the time were maintained in the same cage as their controls.

The method of operation here used gave less than 10 per cent mortality—a result much more favorable than any thus far reported by others. On the other hand, owing to the occasional crushing of the pineal body, parts of it may be left behind, resulting in an incomplete operation. This possible condition was systematically controlled at the end of the experiment by sectioning the brain from the region of the pineal. The presence of bits of pineal substance was in this series always associated with a characteristic growth behavior—to be discussed later.

TABLE 2

Comparison of tests and controls at 85 days

11 males

	TESTS			CONTROLS		
	Final body length	Body weight		Final body length	Body weight	
		Initial	Final		Initial	Final
	mm.	*grams*	*grams*	*mm.*	*grams*	*grams*
Litter 1	190.0	32.5	174.0	180.0	31.0	153.5
	188.0	31.5	170.0	180.0	31.0	153.5
Litter 2	192.0	24.0	184.0	186.0	21.5	161.0
	194.0	23.0	190.5	186.0	21.5	161.0
Litter 3	166.0	19.5	105.0	144.0	20.0	76.1
	175.0	21.3	136.0	144.0	20.0	76.1
	175.0	19 0	138 0	144 0	20 0	76 1
Litter 4	194.0	31.5	189.0	182.0	31.0	159.2
	196.0	32.0	194.0	182.0	31.0	159.2
Litter 5	196.0	34.5	206.5	190.0	32.0	176.0
Litter 6	190.0	22.0	169.0	181.0	21.0	145.0
Averages	186.9	26.4	168.7	172.6	25.4	136.0
	+8.3%	+4.0%	+24.2%			

Effects of the removal of the pineal body from albino rats at 20 days of age. 1. On body growth: In tables 2 and 3 the data according to sex for the tests and controls are given with averages and also the average values for the initial body weight at 20 days.

The data show that at 85 days, i.e., 65 days after operation, the test rats are larger than the controls in length by 8.3 per cent in the males and 5.4

per cent in the females, and in body weight by 24.2 per cent in the males and 17.7 per cent in the females.

Both the tests and controls of each sex were weighed at ten periods during the 65 days following operation and the record of growth thus obtained is given in figures 1 and 2. The graphs show that the more rapid growth of the tests begins at least a few days after the operation and continues to the end of the experiment.

Thus in both sexes of the albino rat accelerated body growth follows removal of the pineal body at 20 days of age.

TABLE 3

Comparison of tests and controls at 85 days

10 females

	TESTS			CONTROLS		
	Final body length	Body weight		Final body length	Body weight	
		Initial	Final		Initial	Final
	mm.	*grams*	*grams*	*mm.*	*grams*	*grams*
Litter 1	187.0	22.5	169.0	181.0	23.0	139.0
Litter 2	184.0	32.0	160.3	179.0	29.5	134.0
	182.0	29.0	154.0	179.0	29.5	134.0
Litter 3	175.0	18.0	145.0	164.0	18.5	125.0
	178.0	19.0	148.0	164.0	18.5	125.0
Litter 4	191.0	27.0	187.5	172.0	24.0	156.0
Litter 5	195.0	34.8	175.0	182.0	34.0	156.0
Litter 6	178.0	21.5	143.5	168.0	20.0	116.5
	176.0	20.0	138.0	168.0	20.0	116.5
	175.0	22.0	132.0	168.0	20.0	116.5
Averages	182.1	24.5	155.2	172.5	23.7	131.8
	+5.4%	+3.0%	+17.7%			

In this connection it may be noted that if one refers to my record for the growth of the pineal body on age (Izawa, 1925, chart 2) it appears that the pineal body was removed at the beginning of its phase of rapid growth (20 days) and that the end of the experiment (85 days) fell just before the close of this phase.

When remnants of the pineal body were found in the brain at the end of the experiment, the operation was recorded as incomplete. The data for these incomplete cases are presented in table 4.

Table 4 shows that in the incomplete group growth was retarded both

in body length and body weight. The values for retardation are statistically valid in the case of the males but not in the case of the females.

Thus the presence of remnants of the pineal body prevents the appearance of those responses which are given by the test rats.

This result is of interest in several ways. Undoubtedly the remnant of the pineal body differed in size in the different rats, but on this point I am unable to report. The operation itself would tend to stimulate the tissues in the region of the pineal, including the remants of the pineal and so under these conditions a fraction of the organ produces as much effect (or more) as the entire organ. The state of affairs seems to be similar to

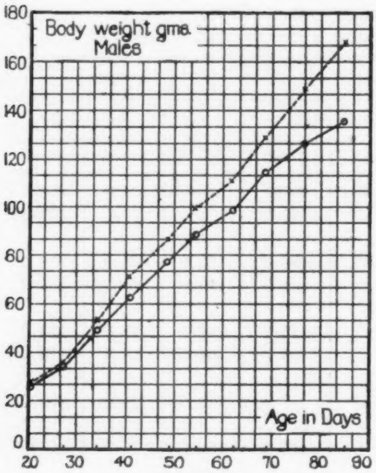

Fig. 1

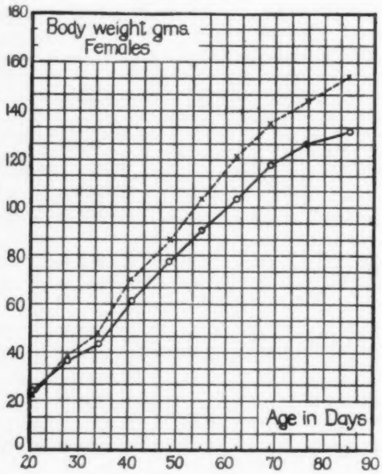

Fig. 2

Fig. 1. The body growth in grams on age of the male control and pinealectomized groups. Albino rat. ○ = control; × = pinealectomized.

Fig. 2. The body growth in grams on age of the female control and pinealectomized groups. Albino rat. ○ = control; × = pinealectomized.

that of the action of remnants of the thyroid gland after parathyroidectomy. I am indebted to Dr. F. S. Hammett for the following information on this point. When the thyroid is left after parathyroidectomy in the albino rat, the effects of removal of the parathyroids are conspicuous. When the entire thyroid is removed with the parathyroids these effects characteristic of parathyroid removal are much less evident or absent. Should, however, a small remnant of the thyroid be left in this latter operation the effects are similar to those found when the entire thyroid is present. Here, then, a small remnant of the thyroid gland brings about effects similar to those appearing when the entire gland is present. These results in the

case of the parathyroids appear to be analogous to those which we have found for remnants of the pineal body. In view of this effect of small remnants it is possible that some of the reports on the absence of accelerated body growth after pinealectomy are due to incomplete removal.

In this series of rats, therefore, remnants of the pineal body serve to hold body growth to the normal or even to retard it.

On the weights of the organs: Since at the time of killing the test rats are larger than the controls they would be expected to have organs absolutely

TABLE 4

Comparison of "incomplete" with controls at 85 days

	INCOMPLETE		CONTROL	
	Body length	Body weight	Body length	Body weight
	mm.	*grams*	*mm.*	*grams*
5 males				
Litter 7	175.0	134.5	180.0	153.5
Litter 8	175.0 184.0	150.0 158.0	192.0 192.0	183.0 183.0
Litter 9	185.0 170.1	145.7 129.0	185.0 185.0	161.0 161.0
Averages	177.6 95.1%	143.4 85.2%	186.8	168.3
4 females				
Litter 7	163.0	116.5	168.0	116.5
Litter 8	185.0	155.0	182.0	156.0
Litter 9	186.0 187.0	158.0 154.0	193.0 192.0	167.0 167.0
Averages	180.2 98.2%	145.9 96.2%	183.5	151.6

heavier than those of the controls. Such is the case. We desire to learn, however, whether in the tests there are any deviations in the relative weights of the organs on body weight, which might be related to the removal of the pineal body.

To determine this it is necessary in the first place to compute the weights of the organs in the controls when their body weights have been raised to those of the tests. This was done in the following way. Using the table for the organ in question as it appears in *The Rat* (Donaldson, 1924) the percentage increase in the weight of the organ from the body weight of the

controls (here, for males, 136 grams) to the body weight of the tests (here, for males, 168.7 grams) is calculated. For the brain, to take that as an example, this is found to be (loc. cit., table 144) 3.2 per cent. The observed brain weight (1.680 grams) in the controls is therefore raised by this percentage (3.2 per cent). This gives 1.7338 grams as the brain weight of the controls when the body weight is increased from 136 grams, the observed value, to 168.7 grams, the value for the tests. By this procedure the data are brought into such a shape that the values for the tests can be properly compared with those for the controls and the relative weights of

TABLE 5

Weights of organs: Percentage deviations of weights in tests from those in controls after correction for body weights

ORGANS	PERCENTAGE DEVIATIONS PLUS (+) OR MINUS (−)			
	Males		Females	
	+	−	+	−
Brain	1.8		0.8	
Spinal cord		8.2	1.4	
Kidneys	0.3			5.0
Spleen		17.3		0.4
Lungs		12.4		1.9
Heart		3.0		4.2
Adrenals		2.4		3.5
Pancreas		4.6		6.3
Thyroid		18.5		2.9
Liver	12.0		4.6	
Submaxillary	7.6			9.2
Only the deviations below this line are statistically valid.				
Hypophysis	0	0		14.8
Ovary			26.1	
Uterus			13.3	
Testes	21.3			
Epididymis	24.3			
Eyes		5.3		7.3

the organs in the tests determined. In this instance the brain weight as observed in the tests is 1.7658 grams or 1.8 per cent above that computed for the controls. For each one of the 15 organs in each sex, the same procedure was followed. The percentages by which the organs in the tests differ (plus or minus) from those in the controls are given in table 5.

On inspection, table 5 shows that of the 15 organs to be considered for each sex, the minus deviations occurring in both sexes are six in number and the plus deviations are five, thus leaving four which are discordant—

plus in one sex, minus in the other. This distribution of the deviations does not in itself furnish any information of value.

Everything depends on the statistical validity of these deviations. To determine this the probable error of the mean for each organ in controls and tests of both sexes was computed and then the probable difference of means (controls and tests) was found by the aid of the formula P.D.M. = $\sqrt{E^2 + E'^2}$. A difference between the means equal to at least three times the probable difference of the means is necessary to make the observed difference significant.

The data for the first eleven organs in table 5 do not stand this test, i.e., the observed differences are less than three times the probable difference of the means in each case. These deviations are, therefore, without significance here.

In the case of the last three organs for the males and the last four for the females, the differences are, by the foregoing tests, found to be significant, and call for comment. It is only in the female that the hypophysis is affected—being retarded in its growth. Previous observations bearing on this result are those of Hatai (1915) which show some enlargement of the hypophysis in spayed females and the determination by Hammett (1925) of some (slight) correlation in weight between the ovaries and the hypophysis. In view of Hatai's results and Hammett's determination it seems possible that the overgrowth of the ovaries and associated organs, may have acted to retard the growth of the hypophysis.

In the case of the eyeballs, though the retardation is slight, it is nevertheless valid and appears in both sexes. Whether here we are recording merely the effect of a traumatic disturbance of the occipital lobes of the brain or whether there is still some connection of the region of the pineal body with the optic apparatus that brings about this result, cannot now be determined.

These two effects of pinealectomy, on the hypophysis in the female and on the eyes in both sexes, have not been previously recorded.

The overgrowth of the gonads and associated structures[1] after removal of the pineal body have been noted by others, and in this instance the overgrowth is very marked.

It appears, then, that in the albino rat, pinealectomy causes an overgrowth of the gonads and some associated structures and a retardation of

[1] In the male, weighings were made of the epididymis (recorded in table 5) and also of the seminal vesicles, prostrate gland and Cowper's gland. All of these three organs had in the tests about double the weight which they had in the controls. In the absence, however, of sufficient data for these organs in the normal rat, it was not possible to determine whether this apparent overgrowth was significant. For this reason these organs have not been discussed in detail or recorded in an table.

the growth of the eyes in both sexes, and in the female, a retardation of the growth of the hypophysis.

Discussion. Pinealectomy has been practised on mammals, birds and amphibia. Here we shall consider only the first two groups.

Mammals. Rat: Foà, Horrax.

Guinea pig: Horrax, Clemente.

Rabbit: Exner and Boesse, Clemente, Sarteschi.

Dog: Dandy, Sarteschi.

Birds. Chicken: Foà, Izawa, Zoia, Clemente, Bardertscher, Christea.

The observations by others may be considered according to the species and the age of the animal used. We begin with the albino rat.

Albino rat. Foà (1914) reports on four albino rats successfully operated when young. There was one female and three males. His method: Sinus and veins ligatured and pineal then picked out. The female showed no effects. The males grew more rapidly at first, but the difference between them and the controls was soon abolished. The testes were enlarged.

Horrax (1916) removed the pineal body successfully from a number of young rats but an epidemic in the colony killed most of them before they could be examined. An overgrowth of the testicles was noted in but one male.

His method was ligature of the sinus: the pineal then pulled out.

Kolmer and Loewy (1922) operated with a fine thermocautery on rats weighing 30 to 50 grams. The authors conclude that pinealectomy does not affect growth. The data for their three groups are, however, +6 per cent, −1.7 per cent and +9.3 per cent respectively for the body weights of the tests.

This comprises the previous observations on pinealectomy in the young rat. Contrasted with the foregoing, my own observations show in the test albino rats (11 males and 10 females) accelerated body growth in both sexes up to 85 days—the end of the experiment. Also accelerated growth, relative to the body weight, in the gonads and some associated sex organs of both sexes.

On the other hand, there is retardation in the growth of the eyeballs in both sexes and of the hypophysis in the female. In the male the hypophysis is not affected.

There was no valid modification in the weights of eleven other organs which were examined (table 5), i.e., they had grown in normal proportion on body weight. We agree, therefore, with Foà in finding accelerated body growth and with Foà and with Horrax in finding an overgrowth of the testicles, though the positive responses reported by both of those authors are less well attested than our own.

Guinea pig. Passing to the other rodents which have been examined there are first the findings in the guinea pig. Horrax (1916) working with young guinea pigs pinealectomized at 2 days to 7 weeks, found in operated males growing to puberty, no difference in body weight when compared with controls but, on the other hand, there appeared to be accelerated growth in the testicles and seminal vesicles. Females bred earlier.

Method: Sinus ligated and pineal pulled out. On two males and one female guinea pig, Clemente (1923), using the method of Foà, reported an overgrowth of the body in all and of the testes in the two males.

Rabbit. In young rabbits, Exner and Boesse (1910) destroyed the pineal body with a thermocautery and carried six out of 95 operated animals to a point at which the development of the genital organs could be examined. The growth of these was not affected by the operation.

Sarteschi (1913) worked on rabbits. The testicles enlarged. Body overgrowth. Method of Lomonaco: ligature of sinus, temporary occlusion of carotids, pineal plucked out.

Clemente (1923) obtained on one male rabbit, results similar to those of Sarteschi (1913).

Foà (1912) was unsuccessful with young rabbits as the experimental animal and Horrax (1916) had a similar experience.

These records show for the other two rodents, the guinea pig and the rabbit, only a slight positive response in the gonads and body growth of the male guinea pig (Horrax, 1916, Clemente, 1923) and early breeding in the female (Horrax, 1916), and in the rabbit some body overgrowth and enlarged testes (Sarteschi, 1913; Clemente, 1923), otherwise they give no support to the results which we have gotten in the albino rat. The several reports further suggest that the rabbit is not well suited for this operation.

Dog. Turning to a mammal of another order, the dog, and limiting ourselves to the observations on puppies, Dandy (1915) reports no changes in the sex organs or sex behavior, nor changes in the other endocrine glands after removal of the pineal body from puppies. Dandy made an elaborate study of the technique for removal of the pineal, which was plucked out.

Sarteschi (1913) worked on very young dogs and reports enlarged testes; one dog overgrown in body weight.

For comparison with my own results, those from the rat, other rodents or carnivora would seem to have suitability in the order named, and the comparison might end here. But there are in addition a number of observations on birds, represented by the chicken, and also on amphibia-frogs.

Chickens. Foà (1912) was able to obtain 15 chickens which survived operation at from 11 to 25 days of age. For the first 2 or 3 months they were retarded in body growth but later grew better. In the cocks, eight to eleven months after operation, there was found a considerable over-

growth of the testicles and combs. No corresponding changes after the initial retardation could be observed in the hens. These results were confirmed on 7 chicks (2 cocks, 5 hens) in 1914.

Christea (1912) reports on 12 young cocks surviving operation. Body growth was not modified but the growth of the testes and of the general secondary sex characters was distinctly *retarded*.

Zoia (1914) reported that when the pineal body is removed from chicks at 15 to 30 days of age there is overgrowth in the testes—no similar response occurred in the female.

Urechia and Grigoriu (1922) kept 2 cocks alive for 8 months after operation and report rapid growth following the first retardation. The hypophysis was also found to be three times as large in the tests as in the controls.

Clemente (1923) found in chickens, after operation, an overgrowth of the body and of the testes and secondary sex organs in the cock.

In his experiments on single-comb white leghorns, Bardetsher (1924) has carried through 5 cockerels and 6 hens from operation at 16 days, to over 200 days of age and followed their growth in body weight. The operation consisted in opening the skull and plucking out the pineal body with a fine forceps. The test animals of both sexes grew somewhat less well than the controls, except that the growth of the hens at 185 to 204 days was a shade better than that of the controls. Those incompletely operated grew sometimes better and sometimes less well than the controls. In the test cockerels the weight of the testes is greater than in the controls, and the same is true for the combs, when the data are corrected for body weight. The author, however, is not inclined to regard these differences as significant.

I have made two studies on the chicken (Izawa, 1922, 1923). In the first study there were 2 cockerels and 1 hen and in the second, 8 cockerels and 3 hens, examined. The pineal body was plucked out with a forceps. The operation was made at 24 to 35 days and examination at about 200 days or somewhat later. The results in both series were similar. The tests grew more rapidly than the control. The gonads in both sexes and secondary sex organs were all enlarged in the tests, while, on the other hand, the growth of the eyes was retarded in both sexes. The weights of the other organs, 13 in number, including the ductless glands, showed no peculiarities in the tests, though some of the ductless glands appeared to be modified histologically. Sexual behavior also was precocious in these test chickens.

When these observations on the chicken are collated it is seen that although there are wide differences in the results obtained by the individual investigators yet there is a body of evidence which indicates that when the pineal body is removed the body, gonads and secondary sex organs show

accelerated growth. Taken as a whole, the responses in the chicken are more definite than in the mammal.

On amphibia (frogs) there have been studies by Adler (1914) and Hoskins (1919) but the results were largely negative.

The limitations of the present study make it inappropriate to consider the results of feeding or transplantation of the pineal body or the clinical observations on the variations in size or in the incidence of disease involving it. References to this literature are found in Bardetscher (1924) and in a very complete form in Krabbe (1923).

Limiting the further discussion to the reports on the rodents, it is plain that the rabbit is a difficult animal for the study of pinealectomy and has not yielded any positive results. It belongs to a different sub-order of rodents than do the guinea pig and rat. The observations on the albino rat by Foà (1914), Horrax (1916) and Kolmer and Loewy (1922) are so fragmentary that they give no real support to our own results. Those by Horrax (1916) on the guinea pig furnish only the accelerated growth of the testes and sexual precocity in the females while Clemente's observations (1923) add body overgrowth.

Extending the comparison, it appears that the results most similar to those found by us in the albino rat are those we were able to obtain with the chicken, as already presented.

Against any wide generalization as to function, are the results of Dandy (1915) on the dog, in which the operation was done with great care. On the other side Sarteschi (1913) reports enlarged testes and some body overgrowth. If we limit our survey to mammals and the chicken, it appears that overgrowth of the testes is most commonly reported. This is a matter relatively easy to determine. Overgrowth of the ovary is not reported except by me in the albino rat and the chicken. This determination is more difficult to make. Body overgrowth is sometimes reported as absent but this is a result which depends largely on the conditions under which animals were kept and there are difficulties always present in maintaining these conditions at a high level.

In view of the somewhat divergent results which we have presented, a word in defense of our findings is in place.

In general, the material used and the conditions maintained during the experiment were superior to those usually found and probably to those available to the other workers on the rat and guinea pigs. Our strain of rats was well established and vigorous and the conditions of the cage life and food unusually well arranged and standardized. These are conditions of great importance in the determination of the post-operative growth. Further, in handling the observed data there is available for the albino a series of reference tables by the aid of which the bodily proportions can be determined and the observed values for the weights of the organs reduced

to a standard body weight—here that of the test. Moreover, the postnatal growth in the weight of the pineal body is known (Izawa, 1925). Finally for each series of data the probable error of the mean has been determined and this followed by the determination of the probable difference between the averages for the tests and controls, in each instance. Mean differences less than three times the probable difference are considered as insignificant. In view of the circumstances surrounding the investigation the following conclusions seem to be warranted.

CONCLUSIONS

In the albino rat:

a. The method of operation yielded a relatively low mortality: less than 10 per cent.

b. Operated controls showed that the first part of the operation was without effect on the body growth.

c. Test albino rats of both sexes showed accelerated body growth between the age of operation, 20 days, and the age of killing, 85 days.

d. In the testes and epididymides of the male there was accelerated growth and in the ovaries and uterus of the female growth was also accelerated. But there was a retardation of the growth of the eyes in both sexes and of the hypophysis in the female.

e. When the removal of the pineal body was "incomplete" growth of the body or of the sex organs was not accelerated; indeed, in the male they appeared to be retarded.

f. In general, complete removal of the pineal body is followed in the albino rat by accelerated growth in the body weight and in the sex organs *in both sexes*. Normally, therefore, it serves to retard these growth processes in the albino rat. The retardations in the growth of the eyes in both sexes and of the hypophysis in the females after operation, may be secondary effects.

g. Corresponding studies on the albino rat by others support these conclusions only in part, but at the same time they were less complete and were made under less satisfactory conditions.

h. The results which are most nearly in agreement are those obtained by me on the chicken (Izawa, 1923).

i. The most serious evidence against a generalization in respect to the functions of the pineal body is furnished at present by the observations of Dandy (1915) on the dog.

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AN EXPERIMENTAL STUDY OF THE EFFECT OF LOW ASH FEEDING ON THE GROWTH OF THE ALBINO MOUSE IN SUBSEQUENT PERIODS ON A COMPLETE DIETARY, WITH SPECIAL REFERENCE TO THE ECONOMY OF FOOD CONSUMPTION[1]

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The experiments herein described were planned to study the economy of food in alternating periods of growth and suppression of growth by means of the limitation of the ash constituents of the diet. It was the intention to compare the daily food consumption during the suppression periods and in the subsequent periods of growth with those shown in an earlier experiment, published with Mendel in 1918, in which growth was suppressed by a quantitative limitation of an adequate diet. In this instance, as in the earlier experiment, the albino mouse was used as the subject. Records of the daily food consumption during the low ash periods and in the periods following the restricted diet have been made and compared with the food consumption of normally growing mice from the age of weaning, 22 days, to the age of 52 days. Body weights taken every third or fourth day have also been compared with those of the controls throughout the period of growth. It was thought that this study would contribute to our knowledge of the relation of growth to food consumption in any period of accelerated growth which might occur following a suppression by a low ash dietary. It was hoped, also, that it would add to our knowledge of the cost, in total food, of maintenance and growth when growth is completed after being suppressed by this method.

Plan of investigation. The mice were placed in individual cages at the age of weaning, 20 to 22 days. The sanitary care of cages, food and water cups, and the regulation of room temperature adopted for the previous experiments were practiced. Food made up from the formula used in the earlier experiments was used for the control animals.

The food contained:

	Grams
Skimmed milk powder	20
Casein	24

[1] The data for this study were collected at the Kansas State Agricultural College during the years 1920 to 1923.

Starch	20
Salt mixture[2]	4
Butter fat	32
Yeast (Torula)	2

By analysis of a mixed sample prepared at several different times the composition was found to be:

	per cent
Protein (N × 6.25)	31.3
Fat (ether extract)	29.3
Carbohydrate	30.3
Ash	4.6
Water	4.5

The yeast was added, 2 grams to each 100 grams of the food paste, as previous experience had shown that it would provide an adequate amount of vitamin B and would secure regularity of eating. For the experimental animals the salt mixture was omitted and the starch was increased to 24 grams per 100 grams of food.

The estimated composition of the low ash dietary with the yeast included was:

	per cent
Protein	31.3
Fat	29.3
Carbohydrate	34.1
Ash	0.7
Water	4.6

As derived from the usual factors, the energy value of the two foods differed but slightly, that of the complete food being 5.2 calories and that of the low ash food being 5.3 calories per gram.

Distilled water was furnished all animals on the low ash diets throughout their lives and all other animals for the period of growth. In the tests with groups VII, VIII, IX, and X, yeast was withheld in order to observe the effect upon food consumption. The results for the entire study are shown for the different periods for each sex. As accelerated growth was not evident in one generation, a few tests were carried into the second and third generations until reproduction failed or until the malnourished females devoured their young at birth.

Normal growth and daily food consumption of controls from the ages of 22 to 52 days. About two months are required for white mice to reach adult proportions and sexual maturity. For this reason the study of the controls in the previous experiment was extended to the 62nd day. In the

[2] Röhmann's salt mixture. From Osborne and Mendel: Carnegie Inst. of Washington, 1911, Publ. 156, I, 32. Quoted by Thompson and Mendel: This Journal, xlv, no. 4, March 1918, p. 433.

present study it was thought unnecessary to continue it beyond the 52nd day as by that time the steep part of the curve had been established and growth had not shown any impairment. The body weights of all males used for the averages from which the curve was drawn are given in table 1, those for the females in table 2. Chart 1 shows a comparison of the curves from these averages with the curves reported in the previous experiment.

The mice used in the groups of controls averaged lower in body weight

TABLE 1

Body weight at age indicated in days. Males

MOUSE NUMBER	AGE IN DAYS										
	22	25	28	31	33	35	39	42	46	49	52
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
2	8.7	9.1	11.9	13.4	16.0	17.1	18.2	18.4	20.0	21.9	22.1
3	9.8	10.2	13.1	15.0	16.7	17.6	20.0	20.1	20.4	21.8	
4	9.0	9.5	12.2	13.9	15.8	17.5	20.0	21.6	22.5	24.6	23.0
6	7.4	10.6	13.7	16.2	17.4	18.5	20.3	21.9	22.4	22.6	
10	9.0	9.8	12.6	15.5	16.8	17.6	18.9	19 7	20 7	20.8	
13	10.4	10.2	12.9	15.8	16.8	17.8	19.7	19.8	19.8	19.9	
19	9.1	9.4	11.2	13.7	15.0	16.4	18.0	18.8	19.0	19.4	
Average...	9.0	9.8	12.5	14.8	16.4	17.5	19.3	20.0	20.7	21.6	22.5

TABLE 2

Body weight at age indicated in days. Females

MOUSE NUMBER	AGE IN DAYS										
	22	24	28	31	33	35	39	43	46	49	52
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
1	8.7	8.5	10.5	13.0	14.5	15.6	17.4	18.5	18.9	19.5	19.7
11	7.3	8.9	10.7	12.5	13.2	14.0	14.8	15.8	15.9	15.9	
14	9.3	9.1	12.2	14.0	14.2	15.2	16.5	17.2	18.6	18.4	
15	8.7	8.3	11.2	11.5	12.9	13.5	14.5	15.4	16.3	16.7	
16	9.2	9.1	12.3	14.3	14.4	14.7	14.7	16.0	16.3	16.4	
21	7.7	8.3	11.6	13.8	14.2	14.4	15.3	16.2	16.4	16.7	
22	8.4	9.0	11.1	13.7	14.4	14.9	15.2	16.0	16.0	16.1	
Average...	8.5	8.7	11.4	13.2	14.0	14.6	15.5	16.4	16.9	17.1	

at weaning than did those previously reported upon, but, by the close of the study, the males had reached or exceeded the average of the first series. On chart 1 the entire group of males is shown in curve 2, figure 1, and the two most rapidly growing individuals, nos. 4 and 6, in curve 1. The females are represented on the same chart in figure 2. Curve 6 for the entire group of females runs parallel with, but somewhat below, the standard curve 7. It is, however, considerably above the curve found by Judson

(1916) who reported the average weights from which curve 8 was drawn. Two of the females, nos. 1 and 14, grew beyond the average curve 6 as shown in curve 5. The rate of increase in body weight over each preceding record for both males and females is approximately the same as in the first experiment up to the 45th day. For the males the gain per cent for the entire time is a little greater than for the first series.

The food consumption, table 3, shows about the same averages daily as in the earlier experiments, although the maxima are slightly lower and

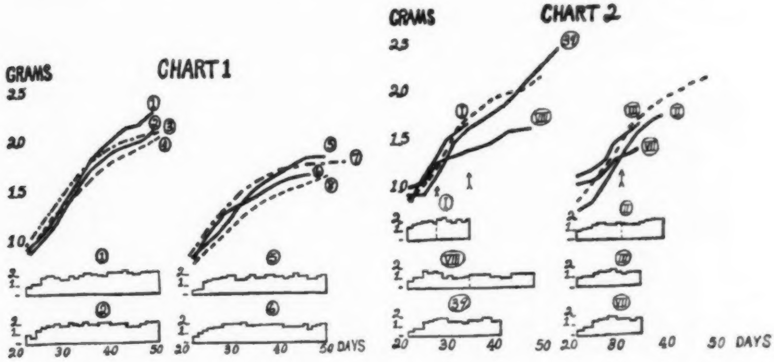

Chart 1. Curves previously reported; ------ Judson, -.-.-. Thompson and Mendel.

Fig. 1. Control males. Curve *1*, averages for nos. 4 and 6; curve *2*, average for group of 7 males. Food chart 1, average consumption of nos. 4 and 6; food chart 2, average consumption for group.

Fig. 2. Control females. Curve *5*, average for nos. 1 and 14; curve *6*, average for group of 7 females. Food chart 5, average consumption for 1 and 14; food chart 6, average consumption for group.

Chart 2. Curve ------, average for controls from curve 2, chart 1.

Fig. 1. Males, I, low ash 6 days, complete food 7 days. Food chart I. VIII, low ash with no yeast 26 days. Food chart VIII. 39, Mouse 39 on low ash 52 days. Food chart 39.

Fig. 2. Males, II, low ash 9 days, complete food 9 days. Food chart II. III, low ash 13 days. Food chart III. VII, low ash with no yeast 13 days. Food chart VII.

the minima higher in most instances. From the correspondences noted it is evident that the controls in the present experiment had an adequate diet from which they ate regularly in sufficient amounts for normal growth.

Food consumption during varying periods on low ash diets, with the effect upon increase in body size and weight. It had been reported by Röhmann (1908) and by Wheeler (1913) that a diet rich in salts as well as in protein was best adapted to the growth of mice. McCollum and Davis (1912–15) and Osborne and Mendel (1913) have shown that a limitation of salts in the diet will check the growth of rats. Judson (1916) found that a reduc-

tion of the salts to 1.75 per cent was sufficient to retard the growth of mice. It should be noted, however, that the food mixture used by Judson did not contain the small amount of yeast which Thompson and Mendel found improved its dietary value. In feeding purified foodstuffs with varying

TABLE 3

Food consumption per day of normally growing mice at successive ages. Age 22 to 52 days

AGE	MALES				FEMALES			
	Number of animals	Food eaten			Number of animals	Food eaten		
		Maximum	Minimum	Average		Maximum	Minimum	Average
days		*grams*	*grams*	*grams*		*grams*	*grams*	*grams*
22	7	1.3	0.4	0.8	7	1.1	0.3	0.8
23	7	1.3	0.4	0.8	7	1.5	0.4	1.0
24	7	2.0	0.6	1.3	7	1.5	1.2	1.4
25	7	2.4	1.1	1.5	7	1.7	1.2	1.4
26	7	2.2	1.1	1.6	7	1.9	1.5	1.6
27	7	2.2	1.1	1.6	7	1.9	1.5	1.6
28	7	2.4	1.5	1.9	7	1.9	1.6	1.7
29	7	2.0	1.3	1.8	7	2.4	1.2	1.9
30	7	2.0	1.3	1.8	7	2.4	1.2	1.8
31	7	2.2	1.9	2.0	7	2.4	1.3	1.8
32	7	2.2	1.9	2.0	7	2.0	1.3	1.7
33	7	2.2	1.4	2.0	7	1.9	1.5	1.7
34	7	2.2	1.9	2.0	7	2.2	1.5	1.8
35	7	2.2	1.9	2.0	7	2.2	1.6	1.8
36	7	2.4	1.8	2.1	7	2.0	1.6	1.8
37	7	2.4	1.9	2.1	7	2.1	1.6	1.9
38	7	2.5	1.8	2.1	7	2.2	1.6	1.9
39	7	2.5	1.8	2.2	7	2.2	1.7	2.0
40	7	2.5	1.6	2 1	7	2.4	1.7	2.0
41	7	2.5	1.6	2.0	7	2.4	1.6	1.9
43	7	2.4	1.7	2.1	7	2.2	1.3	1.8
44	7	2.2	1.5	1.9	7	2.2	1.3	1.8
45	7	2.3	1.5	1.9	7	2.2	1.3	1.8
46	7	2.3	1.5	2.0	7	1.9	1.2	1.6
47	7	2.3	1.5	2.0	7	1.8	1.4	1.6
48	7	2.3	1.6	2.0	7	2.2	1.5	1.9
49	7	2.4	1.6	2.0	7	2.2	1.7	1.9
50	6	2.4	1.8	2.1	7	1.9	1.7	1.8
51	6	2.5	1.8	2.2	7	2.1	1.7	1.9
52	6	2.5	1.8	2.2	7	2.1	1.8	2.0

salt mixtures selected to secure a balance of acid and base, Osborne and Mendel (1918) showed that rats can grow on diets poor in sodium, potassium, magnesium or chlorine, but not on diets poor in calcium or phosphorus. Evans and Bishop (1922) observed only slightly subnormal

growths in rats on low salt diets, but marked retardation in growth of young rats on a diet deficient in potassium was reported by Miller (1923). The literature on malnutrition or subnormal growth of the body as a whole as reported by many observers for different animals has been summarized for the inorganic salts and separately for calcium, phosphorus and iron by Jackson (1925)[3] in his comprehensive treatise on *Inanition and Malnutrition.*

TABLE 4

Body weights and average daily food consumption during varying periods of low ash feeding, followed by adequate diets in two generations of mice. Males

GROUP	NUMBER OF MICE	DAYS OF FEEDING	BODY WEIGHT						FOOD CONSUMPTION						
			Initial weight			Final weight			Average daily intake			Calories estimated		Ash estimated	
			Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Average daily	Per gram body weight	Average daily intake	Per gram body weight
Low ash food plus yeast															
			grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*			*mgm.*	*mgm.*
I	6	6	9.8	8.1	9.0	14.4	11.5	13.3	2.0	1.6	1.7	9.0	0.7	12.0	0.9
II	6	9	8.4	7.5	8.0	14.2	11.2	13.3	1.7	1.1	1.4	7.4	0.6	9.8	0.7
III	3	13	12.6	9.6	11.4	18.0	16.4	17.3	1.7	1.3	1.5	8.0	0.5	10.5	0.6
Complete food															
I	6	7	14.4	11.5	13.3	19.2	14.5	17.1	2.4	2.0	2.2	11.4	0.7	101.2	5.9
II	6	9	14.2	11.2	13.3	18.3	16.7	17.7	2.0	1.6	1.9	9.9	0.6	87.4	5.0
Low ash, no yeast															
VII	7	13	12.5	9.1	10.5	16.5	12.4	14.4	1.8	1.0	1.5	8.0	0.6	9.0	0.6
VIII	4	26	12.5	9.1	10.2	17.6	14.3	16.4	1.7	1.4	1.5	8.0	0.5	9.0	0.5
Low ash plus yeast—second generation															
XI	2	21	11.1	10.7	10.9	21.2	20.6	20.9	2.7	2.1	2.4	12.7	0.6	14.4	0.6
Complete food—second generation															
XIV	3	21	11.6	10.2	11.1	27.3	22.1	23.9	3.2	2.7	2.9	15.0	0.6	133.0	5.6

In the present experiments the low ash dietary contained less than one-sixth of the total ash value of the optimum diet for mice. In spite of the meager daily allowance of essential ash constituents there were no deaths from starvation. There were a few cases of scabs on the tails after long

[3] Jackson, C. M. Inanition and malnutrition, Blakiston, 1925.

continued low ash feeding but, in general, the animals presented a better appearance of health than did those whose growth had been restricted by a quantitative limitation of an adequate diet, thereby seriously limiting their supply of energy.

Statistics of body weights and average daily food consumption for mice on low ash diets. For the males the statistics are given in table 4. Growth

TABLE 5

Body weights and average daily food consumption during varying periods of low ash feeding, followed by adequate diets in two generations of mice. Females

GROUP	NUMBER OF MICE	DAYS OF FEEDING	BODY WEIGHT: Initial weight, Maximum	BODY WEIGHT: Initial weight, Minimum	BODY WEIGHT: Initial weight, Average	BODY WEIGHT: Final weight, Maximum	BODY WEIGHT: Final weight, Minimum	BODY WEIGHT: Final weight, Average	FOOD CONSUMPTION: Average daily intake, Maximum	FOOD CONSUMPTION: Average daily intake, Minimum	FOOD CONSUMPTION: Average daily intake, Average	FOOD CONSUMPTION: Calories estimated, Average daily	FOOD CONSUMPTION: Calories estimated, Per gram body weight	FOOD CONSUMPTION: Ash estimated, Average daily intake	FOOD CONSUMPTION: Ash estimated, Per gram body weight
			Low ash food plus yeast												
			grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*			*mgm.*	*mgm.*
IV	7	6	10.3	8.0	9.2	14.4	10.5	12.6	1.8	1.2	1.5	8.0	0.6	10.5	0.8
V	8	9	8.3	7.8	8.1	14.0	11.0	12.5	1.7	1.2	1.4	7.4	0.6	9.8	0.8
VI	6	13	13.0	9.1	11.1	17.8	14.5	16.9	1.9	1.7	1.8	9.5	0.6	12.6	0.8
			Complete food												
IV	7	7	14.4	10.5	12.6	18.4	12.3	15.9	2.3	1.5	1.9	9.9	0.6	87.4	5.5
V	8	9	14.0	11.0	12.5	17.6	13.6	16.0	2.0	1.6	1.8	9.4	0.6	82.8	5.1
			Low ash, no yeast												
IX	8	13	12.5	8.4	9.9	15.8	12.1	14.1	1.8	1.1	1.5	8.0	0.6	9.0	0.6
X	3	26	12.5	8.4	10.6	17.2	12.5	15.3	1.7	1.4	1.6	8.5	0.6	9.6	0.6
			Low ash plus yeast—second generation												
XII	5	23	12.6	10.3	11.3	21.6	15.5	18.3	2.4	1.5	2.0	10.6	0.6	12.0	0.7
XIII	3	21	11.0	8.7	10.2	15.7	14.3	14.8	1.8	1.6	1.7	9.0	0.6	10.2	0.7
			Complete food—second generation												
XV	4	21	10.7	9.5	9.9	19.6	17.5	18.1	2.6	2.1	2.3	12.0	0.7	105.8	5.8

curves and graphic representations of the daily food intake for the male groups of the first generation appear in the two sets of curves on chart 2. The female body weights and food statistics are shown in table 5, their growth curves and food intake on chart 3. Both male and female groups in the second generation are represented on chart 4. Instances of unusual

growth of one individual in a group have been shown under the individual number, as, no. 39 on chart 2, no. 49 on chart 3, and no. 65 and no. 58 on chart 4. The changes in body weight and average daily food consumption for male groups I and II and for female groups IV and V show very slight influence from the change to the complete food after 6 days or 9 days on low ash diets. The average daily calorie intake per gram body weight remained the same. The intake of ash was materially increased, but there was no acceleration of growth as shown by body weight.

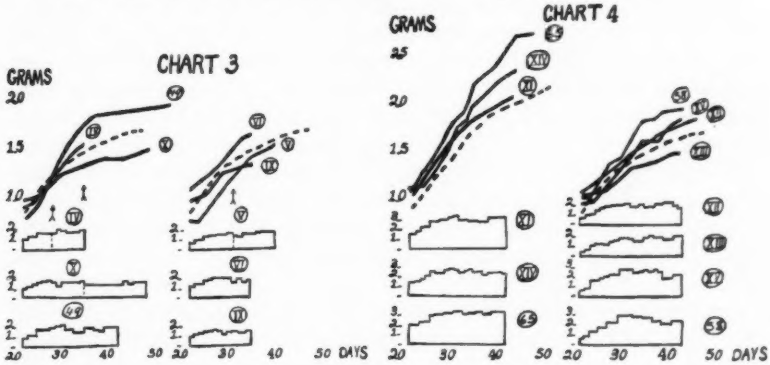

Chart 3. Curve ------, average for controls from curve 6, chart 1.

Fig. 1. Females. IV, low ash 6 days, complete food 7 days. Food chart IV. X, low ash with no yeast 26 days. Food chart X. 49, Mouse 49 on low ash 52 days. Food chart 49.

Fig. 2. Females. V, low ash 9 days, complete food 9 days. Food chart V. VI, low ash 13 days. Food chart VI. IX, low ash with no yeast 13 days. Food chart IX.

Chart 4. Curve ------, average for controls from curve 2 (males) and 6 (females) chart 1.

Fig. 1. Males, XI, second generation on low ash. Food chart XI. XIV, second generation from low ash parents, complete food. Food chart XIV. 65, Mouse 65, one of group XIV.

Fig. 2. Females. XII, XIII, second generation low ash. Food charts XII, XIII. XV, second generation from low ash parents, complete food. Food chart XV. 58, Mouse 58, one of group XV. Food chart 58.

Certain individual differences in food utilization and capacity for growth are noticeable. Mouse 39 belonged to group III. After a few days of irregular eating its food intake averaged higher than did the daily consumption of others in the same group for about five days, after which it was slightly lower. At no time was its food intake equal to the average of group I with which its growth curve may easily be compared. Mouse 49 showed the same ability to grow on a food intake slightly lower, except for the period from the 29th to the 31st day, than the average for group VI to which it belonged. Male 39 and female 49 continued on low ash

diets until the close of the experiment when no. 39 was 400 days old and weighed 31.5 grams, no. 49 at 396 days of age weighed 19.5 grams.

Male group VII and female group IX included the mice in groups VIII and X respectively until the thirteenth day. At that time those growing most slowly in each group were etherized and the remaining ones continued on the low ash food without yeast for another period of 13 days. The slight change in the growth curves is due to the withdrawal of part of the group, not to accelerated growth.

Among the animals of the second generation, male no. 65 was one of a litter of four males and one female produced by no. 49 bred to male no. 39. This was the first litter borne by mouse 49. All members survived and grew on low ash diets after weaning. The female is included in group XII, one male is in group XI. The growth of no. 65 and the two other males is shown as group XIV. This group was fed complete food from weaning on the 21st day. The average daily food consumption, even with the large intake shown for no. 65 alone, did not exceed the average intake for group XI when compared as calories per gram body weight, though the intake of ash was much higher. Female no. 58 was one of a litter of six females, also from male 39, borne by female 43. Female 58 with three of the others compose group XV. This group was fed complete food. The other two females from this litter are in group XIII. From female 43 mated to male 65 there was a litter of three, one male, included in group XI, and two females, included in group XII. All survived the low ash feeding until etherized at the age of 219 days. At the close of the experiment the male of this litter weighed 21.5 grams, the females 20.5 grams and 57.9 grams respectively. Females 43 and 49 lost the reproductive function; no. 43 after bearing two litters, one of six and one of three. Number 49 had litters of five, six and five, but only one from the second and two from the third litter survived. The males retained the reproductive function throughout the experiment, but, after the litters mentioned, the young generated by them, even when borne by control females, died soon after birth. In the statistical tables and the curves, chart 4, shown for the animals of the second generation, male group XIV on complete diet outgrew group XI on the low ash food. Among the females, group XV on complete food exceeded in growth only slightly group XII on low ash but to a marked degree group XIII, also on low ash.

Gains in body weight compared with average daily food consumption. In tables 6 and 7 it is noticeable that for groups having shorter low ash periods when followed by a complete food period, as for male groups I and II and for female groups IV and V, the average total gain per cent was as much or more than shown by the controls when compared from the same initial age. In longer tests with low ash or for groups not changed to complete food, the total gain per cent is invariably less than that made by the

controls. The average quantity of food eaten per day by the experimental animals was often greater than for the controls. Since the vitamins were adequate in amount there were no cases of failure to eat. Ash hunger may have influenced the animals to eat heartily in some instances as in groups of the second generation and in male groups I and III and female

TABLE 6

Gains in weight and average daily food consumption on low ash diets compared with gains in weight and average daily food consumption of controls fed on adequate diets for the same number of days from the same initial age. Males

GROUP NUMBER	NUMBER OF MICE	DAYS ON LOW ASH FOOD	DAYS ON COMPLETE FOOD	INITIAL BODY WEIGHT	GAINS IN BODY WEIGHT: Absolute gains: Average for low ash period or equivalent time	GAINS IN BODY WEIGHT: Absolute gains: Average for period on complete food or equivalent time	GAINS IN BODY WEIGHT: Absolute gains: Average for entire time	GAINS IN BODY WEIGHT: Average total gain per cent	GAINS IN BODY WEIGHT: Average gain per cent during refeeding or equivalent time	FOOD CONSUMPTION: Average daily food intake: For low ash period or equivalent time	FOOD CONSUMPTION: Average daily food intake: For complete food period or equivalent time	FOOD CONSUMPTION: Average daily ash intake: For low ash period or equivalent time	FOOD CONSUMPTION: Average daily ash intake: For complete food period or equivalent time
				grams	*grams*	*grams*	*grams*			*grams*	*grams*	*mgm.*	*mgm.*
I	6	6	7	9.0	4.3	3.8	8.1	90	29	1.7	2.2	11.9	15.4
III	3	13	0	11.4	1.7	4.2	5.9	52	32	1.3	1.7	9.1	11.9
VII	7	13	0	10.5	1.7	2.2	3.9	37	18	1.6	1.1	9.6	6.6
Controls	7	0	13	9.0	3.5	5.0	8.5	94	40	1.3	1.9	59.8	87.4
II	6	9	9	8.0	5.3	4.4	9.7	121	33	1.4	1.9	9.8	13.3
Controls	7	0	18	9.0	5.8	4.9	10.7	119	33	1.5	2.1	69.0	96.6
VIII	4	26	0	10.2			6.2	61		1.5		10.5	
Controls	7	0	26	9.0			12.3	137			1.8		82.8
Second generation													
XI	2	21	0	10.9			10.0	92		2.4		16.8	
XIV	3	0	21	11.1			12.8	115			2.9		113.4
Controls	7	0	21	9.0			11.5	128			1.8		82.8

groups IV, V, and VI of the first generation. Only in male group VII was there a decrease in appetite toward the end of the test. The ash intake for each group has been calculated for the periods indicated in the tables.

Comparison of average total food consumption during period of growth on low ash diets, with and without subsequent adequate diets, with food eaten by controls in the time required to make the same gain from the same initial weight. In table 8 the groups of males in both first and second generations, are

compared in food consumption and in the per cent of food retained as body weight for such periods as were required to make the same gain from the same initial weight. In table 9 the same comparison is made for the females. Group III of the males and group VI of the females had the highest

TABLE 7

Gains in weight and average daily food consumption on low ash diets compared with gains in weight and average daily food consumption of controls fed on adequate diets for the same number of days from the same initial age. Females

					GAINS IN BODY WEIGHT					FOOD CONSUMPTION			
					Absolute gains					Average daily food intake		Average daily ash intake	
GROUP NUMBER	NUMBER OF MICE	DAYS ON LOW ASH FOOD	DAYS ON COMPLETE FOOD	INITIAL BODY WEIGHT	Average for low ash period or equivalent time	Average for period on complete food or equivalent time	Average for entire time	Average total gain per cent	Average gain per cent during refeeding or equivalent time	For low ash period or equivalent time	For complete food period or equivalent time	For low ash period or equivalent time	For complete food period or equivalent time
				grams	*grams*	*grams*	*grams*			*grams*	*grams*	*mgm.*	*mgm.*
IV	7	6	7	9.2	3.4	3.3	6.7	73	26	1.5	1.9	10.5	13.3
VI	6	13	0	11.1	3.4	2.4	5.8	52	16	1.7	1.9	11.9	13.3
IX	8	13	0	10.8	2.0	1.3	3.3	31	10	1.5	1.5	9.0	9.0
Controls	7	0	13	8.5	2.9	3.2	6.1	72	28	1.3	1.7	59.8	78.2
V	8	9	9	8.1	4.4	3.5	7.9	98	28	1.4	1.9	9.8	13.3
Controls	7	0	18	8.5	4.7	2.6	7.5	88	19	1.4	1.9	64.4	87.4
X	3	26	0	10.6			4.7	44		1.6		11.2	
Controls	7	0	26	8.5			8.4	99			1.6		82.8
Second generation													
XII	5	23	0	11.3			7.0	62		2.0		14.0	
XIII	3	21	0	10.2			4.6	45		1.7		11.9	
XV	4	0	21	9.9			8.2	83			2.3		105.8
Controls	7	0	21	8.5			7.9	94			1.8		82.8

average weights at the beginning of the low ash feeding, therefore the weights of these groups were taken for the initial weights in making this comparison. The ages of the other groups indicate the time at which their average weight corresponded to the 22-day weight records of the group for their sex. A gain of approximately 6 grams for the first generation and

TABLE 8

Comparison of average total food consumption during period of growth on low ash diets, with and without subsequent adequate diets, with food eaten by controls in the time required to make the same gain from the same initial weight. Males

GROUP NUMBER	NUMBER OF MICE	AGE	GAINS IN BODY WEIGHT	DAYS REQUIRED FOR GAIN	FOOD EATEN: Low ash period	FOOD EATEN: Complete food period	FOOD EATEN: Average total food intake	FOOD EATEN: Average total ash intake	GAINS IN BODY WEIGHT COMPARED WITH FOOD CONSUMED DURING THE PERIODS IN WHICH THE GAINS WERE MADE
		days	*grams*		*grams*	*grams*	*grams*	*grams*	*per cent*
I	6	25	6.1	9	5.6	13.2	18.8	0.65	32
II	6	28	6.1	6	4.7	5.0	9.7	0.26	63
III	3	22	6.2	8	12.4		12 4	0 09	50
VIII	4	25	5.8	22	30.1		30.1	0 18	19
Controls	7	26	6.3	7		12 7	12.7	0 58	50
Second generation									
XI	2	22	10.0	20	48.1		48.1	0.34	21
XIV	3	22	10.0	14		40.0	40.0	1.84	25
Controls	7	26	10.0	19		37.9	37.9	1.74	29

TABLE 9

Comparison of average total food consumption during period of growth on low ash diets, with and without subsequent adequate diets, with food eaten by controls in the time required to make the same gain from the same initial weight. Females

GROUP NUMBER	NUMBER OF MICE	AGE	GAINS IN BODY WEIGHT	DAYS REQUIRED FOR GAIN	FOOD EATEN: Low ash period	FOOD EATEN: Complete food period	FOOD EATEN: Average total food intake	FOOD EATEN: Average total ash intake	GAINS IN BODY WEIGHT COMPARED WITH FOOD CONSUMED DURING THE PERIODS IN WHICH THE GAINS WERE MADE
		days	*grams*		*grams*	*grams*	*grams*	*grams*	*per cent*
IV	6	25	5.2	9	5.3	11.6	16.9	0.57	31
V	8	29	4.8	10	3.3	15.8	19.1	0.75	25
VI	6	22	5.2	9	15.8		15.8	0.11	33
X	3	25	4.8	22	34.6		34.6	0.21	14
Controls	7	27	5.0	11		16.5	16.5	0.82	30
Second generation									
XII	5	22	7.5	23	46.2		46.2	0.32	16
XV	4	24	7.4	18		45.0	45.0	2.07	16
Controls	7	27	7.1	23		34.6	34.6	1.59	21

10 grams for the second generation of males is shown with the average total food intake and the gain in terms of per cent of food retained as body weight. The calculation of gains in weight in terms of food intake excludes the water which, obviously, enters in considerable quantity into new tissues but for which no measurement can be made. Group II and the controls had reached the ages of 28 days and 26 days respectively. They showed gains in body weight in comparison with food eaten of 63 per cent and 50 per cent respectively. Group III gained at the rate of the controls using one day more to make the gain, while groups I and VIII gained more slowly and, therefore, showed a lower gain per cent of the food eaten during the period. It is noticeable that, while the average daily food intake for group VIII was as high as that of other groups making better gains, there was a very low ash intake due to the absence of yeast in the diet. This may have influenced the utilization of the food. The largest average daily food intake in the groups of the second generation, group XI on low ash food and group XIV on complete food, as compared with that of the controls, is considerably in excess of it. At the age of 22 days the animals in these groups, although reared by mothers grown on low ash diets, averaged the same in body weight as the controls at 22 days of age. Acceleration of growth is shown by group XIV. This group gained in 14 days the same amount as did the controls in 19 days, the gain representing 25 per cent of the food intake for the period while that of the controls was 29 per cent.

For the females the gain in body weight used in the comparison for the first generation was approximately 5 grams, for the second generation, 7 to 7.5 grams. Results similar to those shown for the males were found, in that group X on the diet with no yeast made the lowest gain in comparison with the average total food intake. Two groups, IV and VI, of the first generation made slightly greater gains than the controls, while groups XII and XV of the second generation made slightly accelerated growths. The food intake for these two groups was considerably higher than for the controls.

With the small intake in ash it is apparent that the tissue development must have differed to some degree from the normal in the several groups of mice described. Very thin skull bones with open sutures and delicate long bones were found at autopsy of animals in the low ash groups. The bones were examined and tested to find the relative strength of those from animals on low ash diets, those that were refed after restricted periods, and the controls. The findings in regard to bone structure, bone strength, and body measurements will be given in a subsequent paper. The teeth seemed perfectly formed and sufficiently hard to endure through the time the animals were kept, the longest time being 446 days. There is, doubtless, a high ash retention during the suckling period. Young mice develop

teeth rapidly enough to begin to eat as soon as they can use their eyes and move about the cage. During the third week of life normally growing mice will eat such hard materials as dog biscuit. The mice used in this experiment were fed on a paste food which offered no resistance to the teeth, hence nothing can be said of the strength or durability of the teeth in use on hard materials.

The conservation of ash when the dietary supply is scant may be characteristic of small animals with capacity for rapid growth. Or it may depend upon the other food factors. Bartlett (1916) reported that, for the human infant, a large intake of protein is accompanied by a large retention of salts *if the intake of salts is also high*, while a high fat diet given for a sufficient length of time causes a de-alkalization of the tissues. In the present experiment both protein and fat were high but the ash seems to have been conserved from a low intake. By a slight reduction of the mineral content of goat's milk for kids artificially fed, Telfer and Crichton (1924) found a perceptible degree of osteoporosis. A further reduction was followed by definite signs of malnutrition which were entirely obviated by the addition of an excess of salt mixture roughly similar to the ash of goat's milk. These animals, however, were subjected to experiment from birth. The suckling period is probably more easily affected by a low ash diet than periods after weaning. In the present experiments the first generation of mice was suckled by mothers having adequate diets. The second generation was restricted during the suckling period by the low ash diet of the mothers.

The deficiency of iron might be expected to show in a lowered hemoglobin in the animals that lived for long periods on the low ash diet. Bunge[4] reported experiments done in his laboratory by Hausemann in which, by means of a milk and rice diet, the hemoglobin in young rats was diminished to about half that of normal animals. In rearing mice on the same low iron diet—milk and rice—Schmidt (1912) described the third generation as having both hemoglobin and number of red cells less than the normal. Happ (1922) found that, in rats, well-balanced diets that were poor in iron did not produce anemia in the first generation but a slight anemia sometimes occured in the second generation. It is possible that the high vitamin B in the present experiment favored the retention of iron. Jackson[5] has called attention to the fact that in vitamin B deficiency (beriberi) there is usually marked anemia. Happ, however, did not find anemia in rats as a consequence of deficiencies of either vitamin A or B even when there were severe nutritional disturbances. Haramaki (1922), in feeding

[4] Physiological and Pathological Chemistry, Blakiston's edition, Philadelphia, 1902, p. 379. Quoted by Sherman, Chemistry of Food and Nutrition, Macmillan, 1918, page 292.

[5] Jackson: Inanition and Malnutrition, p. 239.

dogs, found that iron was retained on a vitamin free diet but that, on the addition of vitamins to the diet, the retention of iron was increased *if an excess of iron over that given in the earlier period was fed.*

At the close of the experiment with low ash diets many of the older mice were tested for hemoglobin by use of the Tallquist color chart. Tests were made, also, from controls of the same age, from young growing normal mice and from young adults on a mixed diet. While the lack of accuracy of this method was recognized, it seemed to be the most practicable. A new chart was used after thoroughly drying it. All tests were made within four days so may be considered as correct in relation to each other. The color corresponding to 100 per cent on the chart was not quite intense enough for the blood color of young growing males. For six animals the color was indicated as 100 plus. In young growing females the color corresponded to 100 per cent. In young adults, in both males and females, it was between 90 and 100, in most cases nearly 100. Old stock females that had borne several litters of young had hemoglobin colors between 80 and 90. Control males killed at 446 days of age showed a color slightly below 90. For control females at the same age it was between 80 and 90. Experimental animals were found to have the following color range: males, at 308 to 400 days of age 80 to 90, males of the second generation, at 219 days of age 70 to 80, females, at from 308 to 396 days of age, approximately 80. One very obese female of the second generation had a hemoglobin color of 70 and a body weight of 57.9 grams when killed at the age of 219 days. In no case was there a loss of hemoglobin to half the normal.

For the animals kept for long periods on low ash diets the daily food intake was not recorded after adult size was reached. No animal died of ash starvation although in some cases they ate their own fur from their front legs and chest before being finally etherized. No bedding was used in the cages. Feces were not allowed to accumulate. Some of the mice

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Fig. 1. No. 6, control male. Weight at 22 days 7.4 grams. Picture on 80th day. Weight 30.0 grams.

Fig. 2. No. 1, control female. Weight at 22 days, 8.7 grams. Picture on 80th day. Weight 26.7 grams.

Fig. 3. No. v, young stock male. Picture 30th day. Weight 14.0 grams.

Fig. 4. No. 49, female. Weight at 22 days, 9.2 grams. Picture on 396th day. Weight 19.7 grams. Low ash diet for 374 days.

Fig. 5. No. 39, male. Weight at 22 days, 9.6 grams. Picture on 400th day. Weight 31.5 grams. Low ash diet for 378 days.

Fig. 6. No. 43, female. Weight at 22 days, 9.1 grams. Picture on 396th day. Weight 50.6 grams. Low ash diet for 374 days.

Fig. 7. No. 65, male. Weight at 22 days, 10.2 grams. Picture on 308th day. Weight 27.5 grams. Second generation on low ash diet.

Fig. 8. No. 71, female. Weight at 22 days, 12.6 grams. Picture on 219th day. Weight 57.9 grams. Second generation on low ash diet.

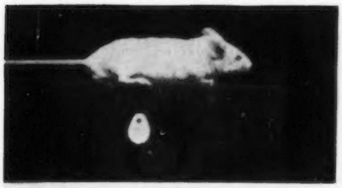

Fig. 1 6

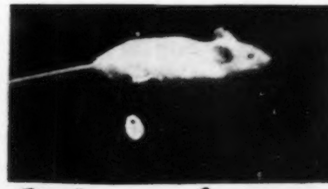

Fig. 2 1

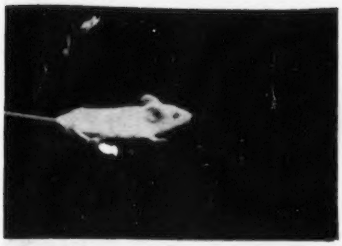

Fig. 3 v

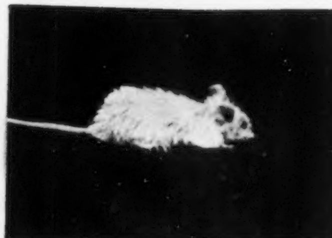

Fig. 4 49

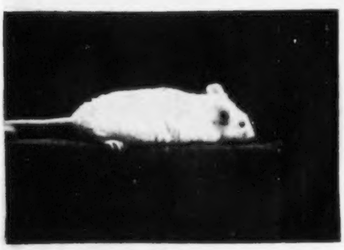

Fig. 5 39

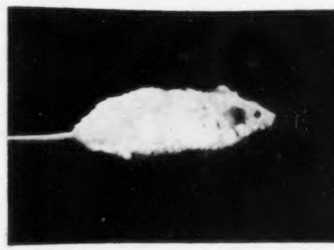

Fig. 6 43

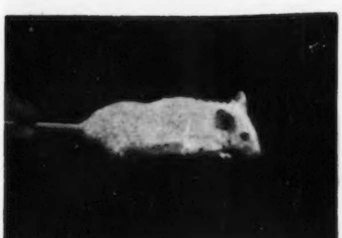

Fig. 7 65

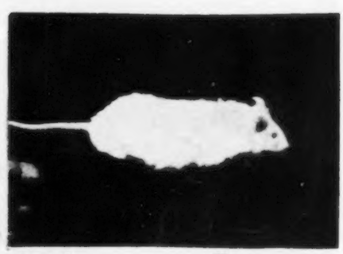

Fig. 8 71

grew fat for a few weeks and then lost weight gradually, remaining at average weight for adults in their old age. Others gained steadily and showed extreme over weight and inactivity. Fatty infiltration was found in all the internal organs besides large quantities of fat in the usual storage spaces. There were a few cases of moist looking fur, but, more commonly, the fur was rather thin and dry and without luster. The animals were usually quiet and easy to handle. They did not need to search for food as they were always given a surplus. They did not show the restlessness which was common to the animals in the earlier experiment in which the total daily quantity of food was restricted.

SUMMARY

On low ash diets containing ample vitamins and protein and of sufficient energy value albino mice grew at the usual rate as indicated by body weight. On the same diet with omission of the yeast which furnished vitamin B and part of the ash, growth was depressed but not entirely arrested. Accelerated growth was not induced by a high ash diet following the period of low ash feeding.

In the second generation accelerated growths were made by the animals on adequate diets. All but one group on low ash diets grew above the normal average rate.

Lowered hemoglobin was observed but, except in the second generation, it was no greater in degree than among old stock animals or old controls. The bones were delicate. Obesity was common in old age and more frequent among females than among males.

Average daily food consumption, when calculated in calories per gram body weight, varied from 0.5 calorie for the most slowly growing animals to 0.7 calorie for the most rapidly growing groups. The average daily ash intake, calculated in milligrams per gram body weight was as low as 0.5 mgm. and 0.6 mgm. for animals that had no yeast in their diet. It averaged from 0.6 to 0.8 mgm. for those on low ash with yeast, while controls and groups refed on high ash diets consumed from 5 to 6 mgm.

As measured by average daily food intake and average total gain per cent, the experimental animals on low ash diets for the shorter periods made approximately the same gains as did the controls in the same number of days from the same initial age. Those on low ash diets for 13 days or longer made slightly lower gains. As judged by the per cent of food retained as body weight, the animals on experiment used more food than did the controls to make the same gains from the same initial weights.

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STUDIES ON CHOLIN AS A MOTOR HORMONE FOR THE ALIMENTARY TRACT

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Twenty-two years ago Magnus (1905) demonstrated that an extract can be obtained from the gut wall having a stimulating action on the tone and motility of isolated segments of intestine. A little later Eriquez and Hallion (1904) showed that an extract of minced dog's intestine injected intravenously caused increased motility of the gastro-intestinal tract of the animals injected. The active principle, they found, was heat-stable and alcohol-soluble. It remained for Weiland (1912) to demonstrate that the active substance investigated by Magnus and by Eriquez and Hallion could be obtained from the living gastro-intestinal tract by diffusion.

Weiland bathed the stomach and small and large intestines of dogs, cats and rabbits in warm Ringer's solution, and each time obtained a substance which stimulated intestinal peristalsis when applied to isolated pieces. He determined that the active principle is stable to boiling and to antiseptics; soluble in alcohol and ether and soluble with difficulty in acetone; and diffusible. He estimated that a whole intestine yielded about 1 mgm. of the active substance, which is further alkaline to litmus, gives the biuret reaction and is precipitated by phosphotungstic and phosphomolybdic acids. He did not succeed in obtaining a chemically pure substance. Weiland showed further that this substance acts on Auerbach's plexus, and that minimal doses of atropin antagonize its action.

Injected intravenously it lowers the blood pressure of rabbits, while in cats, though the drop in blood pressure is more definite, it quickly returns to normal. Two of his dogs showed strong epigastric peristalsis during the intravenous administration, while rabbits receiving daily intravenous doses of the diffusate developed diarrhea without fever or other general symptoms, or pathological findings at post-mortem. From these experiments he concludes that:

> Die Darmwand selbst eine Substanz enthalt, welche den Auerbach'schen Plexus erregt, zeigt, das wir auch für die automatischen Bewegung des Darmes eine chemische Ursache anzunehmen haben.

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Le Heux (1918) repeated Weiland's experiments and not only confirmed his observations, but succeeded in identifying the active principle as choline. He states that "The marked increase of activity imparted to the active fraction after acetylation, in conjunction with the formation of precipitates with phosphotungstic acid and alcoholic solutions of $HgCl_2$ and $PtCl_4$ seems good evidence that the active principle is cholin, or else some analogous substance." The physiological effects of the purified extract injected intravenously compare very well with those obtained by the injection of pure cholin solutions. Acetylation of each causes approximately the same increase in activity—about 10,000 times—upon the isolated frog's heart.

Stimulated by these findings, le Heux (1920) attempted to formulate a theory of hormonic gastro-intestinal control which might explain the variable action of atropin on isolated intestinal strips obtained by various workers and often by the same individual (Magnus, Trendelenburg, Hirz, de Jende, Liljestand and others).[2]

Atropin, le Heux argues, is antagonistic to cholin. Could it not be that the cholin content of the strip examined has some influence upon the action of the added atropin? A non-paralyzing dose of the latter drug will depress or not depending upon the amount of cholin present. If one were to wash the strip for several hours to remove the cholin, then the action of atropin would manifest itself without antagonism; and if its depressing effects depend upon that antagonism, then atropin should be *a*, without effect on the washed strips; and *b*, it should regain its effectiveness upon the readdition of cholin. And this is precisely what does occur.

According to le Heux, then, there is in the intestinal wall during life sufficient cholin to increase the activity of Auerbach's plexus. If this cholin is diminished in amount by oft repreated washings, atropin will cease to act in doses previously adequate, until a concentration is reached when the atropin can act directly upon Auerbach's plexus, but not as an irritant. Very large doses paralyze the nervous centers, nerves and muscle. In the intact animal where there is no cholin depletion, depression is obtained with small doses of atropin.

The question now arises whether these facts permit the classification of cholin as a hormone: the hormone of gastro-intestinal motility. Abderhalden and Paffrath (1925) have confirmed le Heux' findings of free cholin in the gut wall, and support his contention that cholin be considered a hormone.

They injected 150 cc. warm Ringer's solution intra-peritoneally in a rabbit and drained it off an hour later, obtaining 75 per cent. This solution, assayed biologically, was found lacking in cholin. The same gut was taken from the body and suspended in warm Ringer's solution to increase its activity,

[2] See le Heux (1920) for complete literature and discussion.

the other was left undisturbed. The outer solution was changed every few hours and its cholin content determined. Both specimens gave the same total amount after 12 hours—a maximum. But the active segment yielded 90 per cent of its cholin the first 6 hours, the quiescent segment gave 90 per cent from the third to the ninth hours. Their general conclusion is that the amount of cholin at any time is proportional to the activity of the segment, and that these results offer new support to le Heux' contention of the hormone action of cholin.

Abderhalden and Paffrath's experiments do show one thing: that cholin is possibly a by-product of intestinal activity and disintegration. He offers no explanation of his inability to obtain cholin in the intact animal by intra-peritoneal perfusion.

Le Heux (1921) reasoned that if cholin is to be considered a gastro-intestinal motor hormone it ought to have some influence on the intact animal's gastro-intestinal tract. He fed a barium meal to cats, and after determining the average emptying time of stomach and intestine of that animal, injected 10 mgm. cholin intravenously and determined its effect on the digesting gastro-intestinal motility. He reports immediate antral peristalsis following the injection; an increased rate of filling and peristalses of the duodenum; and clearly increased gastric movements after one hour. The stomach emptied 15 minutes more quickly than in the controls, and the barium reached the proximal colon from $\frac{1}{2}$ to $\frac{3}{4}$ hour sooner! From these results he concludes that cholin given intravenously to cats quickens antral peristalsis; increases the rate of duodenal filling; but has no effect on the passage of the meal through the distal colon.

Kühlewein (1921) reported a marked improvement to follow the intravenous administration of 5 to 15 mgm. cholin chloride per kilogram of body weight, in cats whose gastro-intestinal tract had been previously paralyzed by deep chloroform anesthesia lasting two hours. Arai (1922a) observed analogous improvement in cats suffering from iodine peritonitis or from gastro-intestinal paralysis due to laparatomy and purposeful handling of the gut.

Zunz and György (1914), Kühlewein and Arai (1922b) have shown that chloroform or ether anesthesia, or morphine, do not change the cholin content of the alimentary tract and conclude that the paralysis incident to these forms of anesthesia cannot be due to loss of cholin.

Klee and Grossmann (1925) discussing the clinical efficacy of cholin conclude that to be active on the gastro-intestinal tract it must be given intravenously and fairly rapidly, since it is inert when given by mouth, subcutaneously or intramuscularly. They could find no evidence of its efficacy in gastro-intestinal disorders.

Spatz and Weichmann (1924) using cholin hydrochloride (Kahlbaum) in 600 mgm. doses dissolved in 240 cc. warm saline found that the action of

the intravenously administered drug on the alimentary tract as shown by the x-rays, was inconstant, most cases showing no appreciable effect; a few showing increased and a few decreased activity. Their cases were routine post-anesthesia patients.

EXPERIMENTAL. In view of the above reports it was decided to test the effect of cholin HCl on the hunger contractions and digestion peristasis of the dog's stomach as recorded by the modified balloon method of Boldyreff (1904) described by Carlson (1912).

The cholin HCl used was obtained from the Eastman Kodak Co. in one-gram vials in crystal form and used as a 1 per cent solution in saline.

This solution when added to an isolated rat gut preparation showed activity in the concentration of 1 mgm. in 75 cc. of Ringer's solutions. Intravenous injection of 50 to 100 mgm. into a 10-kilo dog under ether narcosis gave a sudden fall in blood pressure followed by a rapid rise to above normal. The solution was made up fresh every few days and remained clear throughout the observations.

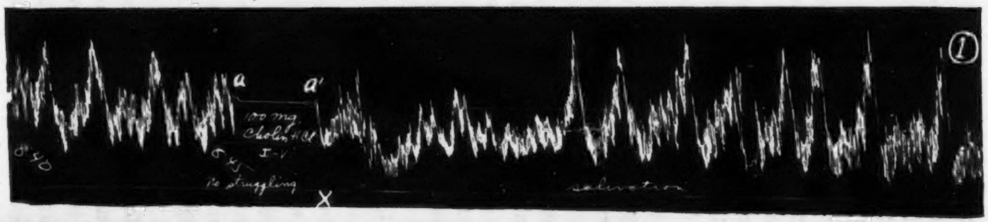
The balloon was passed into the stomach by way of a gastric fistula and no observations were made until the stomach showed good tonus and active hunger contractions, and the wounds had healed. This manner of introducing the balloon was chosen as offering the least possible psychic disturbance to the animal, making possible comparable daily readings and undisturbed oral feedings with the balloon in situ. Except for the operative procedure, the animals received no anesthesia or other quieting measures. They would lie quietly for hours in perfect comfort, and would often fall asleep.

Four healthy male dogs were used, two weighing about ten and the other two about eighteen kilograms.

RESULTS. Two series of observations were made on these animals, during which 5 to 10 mgm. per kilo body weight were injected, during a time from $\frac{1}{2}$ to $1\frac{1}{2}$ minutes depending upon the size of the dose.

I: *a*, The effect of cholin HCl given subcutaneously, and *b*, the effect of cholin HCl given intravenously on the hunger contractions. These injections were given at the onset of, or at the height of, a hunger period, or during a period of complete quiescence.

The results were uniformly negative, the immediate and remote effects upon the motility of the stomach being conspicuous by their absence (fig. 1). The records were followed for from two to four hours consecutively, after the injection, often again after eight and sometimes after 24 hours. Daily intravenous injections caused no demonstrable untoward effects, and no diarrhea followed. The intravenous injections of 10 mgm. per kilo body weight were invariably followed by a general depression, so that a restless, apprehensive animal soon rested quietly for the remainder of the experiment.



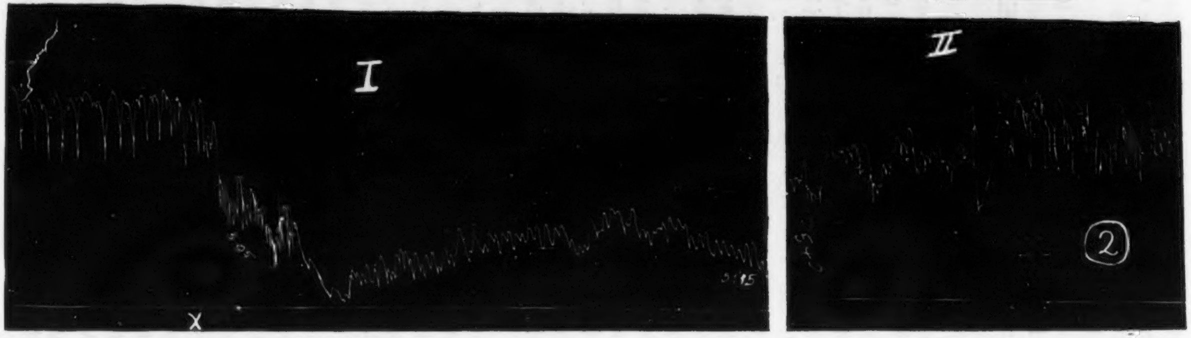


Fig. 1. Dog. Balloon. Water manometer record of gastric hunger contractions. *a-a'*, tube to manometer clamped during insertion of needle in femoral vein. *x*, injection (femoral vein) of 100 mgm. cholin chloride. Showing no definite effect of this quantity of cholin on the gastric hunger contractions.

Fig. 2. Dog. Balloon. Water manometer record of gastric digestion peristalsis after ingestion of 50 grams lean meat. *x*, intravenous (femoral) injection of 900 mgm. cholin chloride. Showing prolonged depression of gastric tonus and peristalsis. *II*, showing return of tonus and peristalsis 35 minutes after the cholin injection.

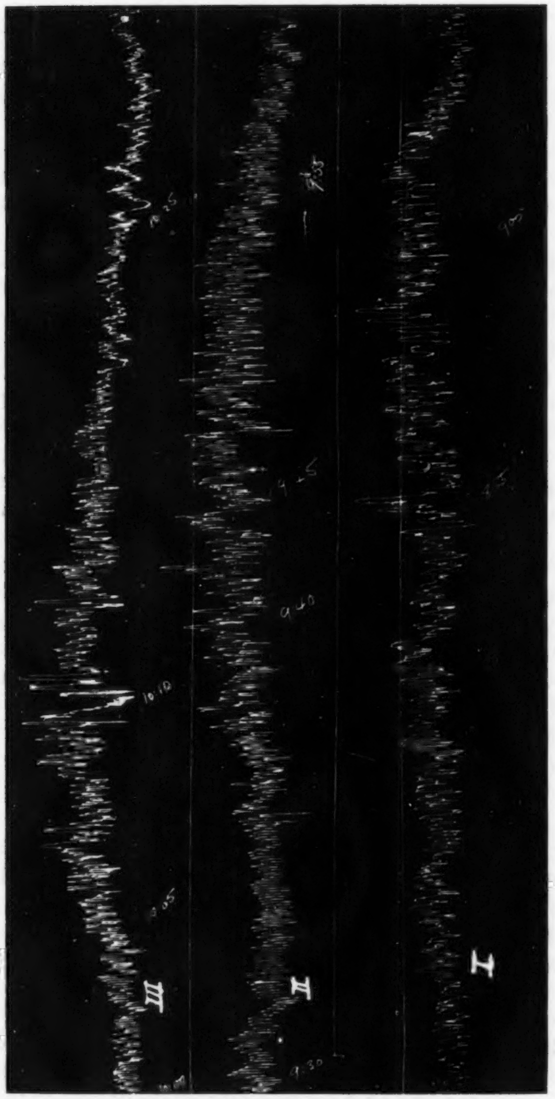


Fig. 3. Balloon. Water manometer record of gastric tonus and digestion peristalsis after 50 grams of lean meat. Dog fed 10 minutes before beginning of tracing *I*. End of tracing *III* is 2 hours after the feeding. Showing long periods of increased gastric tone and digestion peristalsis, alternating with periods of relative depression of tone and quiescence.

Both the intravenous and subcutaneous injections gave salivation, the former marked and prolonged, the latter slight, and as far as could be determined, of short duration.

II. *a*, The effect of cholin HCl given subcutaneously; and *b*, the effect of cholin HCl given intravenously on the "emptying time" of the stomach and the digestive phase of gastric activity. The "emptying time" considered here is that interval included between the disappearance of the hunger contractions due to feeding 50 grams of chopped meat and the reappearance of those waves, the balloon remaining in situ throughout the observation.

The subcutaneous injections of cholin HCl had no effect whatsoever upon the time of reappearance of the hunger contractions, or upon the digestive contractions or tone of the digestive cycle.

The intravenous injection of cholin HCl, however, gave definite positive results when given in doses of 10 mgm. per kilo body weight. Injected immediately after feeding, it depresses the tone and causes the digestive gastric waves to disappear if present, or delays their onset. Administered at the height of digestion it causes a marked depression of tone and a cessation of all gastric motility (fig. 2). The stomach regains its tone and digestive motility from $\frac{1}{2}$ to 2 hours after the injection. There was no vomiting.

During the digestive period it was often noted that the gastric tone of the digesting stomach would vary in the form of a shallow curve the trough of which was void of any activity; the crest being marked by deep digestive waves of short duration and marked frequency. The changes in tone occurred about three times per hour, about one-sixth of the hour being occupied by the decreased phase. These waves of gastric activity during digestion hitherto not described, but which corresponds with the well known rhythmic activity of empty stomach (fig. 3).

SUMMARY

1. A study has been made by the balloon method of the effect of cholin hydrochloride in doses up to 10 mgm. per kilo upon the hunger and digestive phases of gastric activity, in dogs not under an anesthetic.

2. Cholin HCl in 1 per cent solution, injected subcutaneously during hunger, or during digestion, has no demonstrable effect with the method and dosage used, upon the normal activity of the stomach. It also has no effect upon the hunger phase when injected intravenously.

3. Cholin HCl injected intravenously causes a cessation of gastric activity and a marked lowering in gastric tone during digestion; when injected simultaneously with the feeding it causes a marked delay both in the onset of gastric activity and rise of tone of the stomach.

4. The observation of Klee and Grossmann that subcutaneous administration of cholin HCl is ineffective has been confirmed.

5. The cholin HCl used in these experiments when injected into the vein of a dog under ether narcosis in doses of 5 or 10 mgm. per kilo causes a prompt drop in blood pressure which quickly rebounds to above normal.

6. The observations of le Heux, Kühlewein and Arai that intravenously injected cholin HCl causes gastric peristalsis in the cat, could not be confirmed by our method for the dog.

7. A hitherto undescribed periodicity of gastric tone and contractions during digestion has been described.

The writer wishes to thank Professor Carlson for his valuable assistance and criticism in making this investigation possible. He is also indebted to his chief, Dr. F. W. Schlutz, for the opportunity to do this work, and to Dr. H. C. Chang for his assistance in performing the necessary operative procedures.

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THE EFFECT OF VARIATIONS IN PROTEIN INTAKE ON THE ACIDITY OF THE SECRETION OF THE FASTING STOMACH

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In a previous paper the writer (1925) showed that, during observation periods of a single day or less, the acidity of the fasting gastric contents varied mainly in synchrony with the periodic gastric motor activity. It was incidentally mentioned that the average acidity was found to be relatively high on some days while it remained characteristically low on other days. This variation in the average acidity bore no noticable relation to changes in the gastric motor activity but was found to be mainly the consequence of variations in the protein intake. The protein intake was primarily varied in order to study the effect on the complex of sensation or condition which would naturally lead one to eat or to refrain from eating.

Methods. The method used in obtaining data regarding the acidity of the contents of the fasting stomach was described in the paper referred to above. Briefly stated, aspirations of the fasting stomach were usually made about every thirty minutes during the observation periods and the aspirate was promptly returned to the stomach, by way of the tube, after noting the quantity and character and generally withholding only 1 cc. for titration. The observation periods usually began shortly after rising and ended before food, water or any other material was taken. On some days no aspirations were made while on other days from 1 to 32 (during $15\frac{1}{2}$ hours of continuous observation) were made. During the last four months of this study from 2 to 13 (average 6.5) half-hourly aspirations were made daily. The entire study lasted a full year during which over 2000 aspirations were made. The writer was the subject.

The protein intake was varied at first by using the subject's chief source of protein (meat) only two or three times a week but using it to the point of repletion on the protein days. Gradually the periods of protein restriction and of protein realimentation to the point of repletion were lengthened. Food other than protein was also so selected as to accentuate the contrast in protein intake. At the same time an effort was always made to keep the diet otherwise adequate and palatable.

Results. *Preliminary experiments.* As may be seen by table 1, the

average acidity of the fasting gastric contents was lower after a liberal protein intake and usually higher after protein restriction. The effects seemed to be proportional to the protein intake and the length of the

TABLE 1

Showing that the acidity of the fasting gastric contents is generally lower after a liberal protein intake and higher after protein restriction

DATE (1925)	NUMBER OF ASPIRATIONS*	PER CENT FREE HCl			REMARKS
		Lowest	Highest	Average	
February 11	7	0	0.05	0.016	Day after liberal (about 175 grams) protein intake
February 14 to 18	15	0.01	0.13	0.075	After protein restriction (about 25 grams daily)
February 21 to 23	10	0.06	0.14	0.100	After moderate protein intake following restriction
February 24 to 26	17	0	0.13	0.048	After liberal protein
February 27 to March 5	41	0	0.15	0.086	After protein restriction
March 6 to 9	25	0	0.12	0.030	After liberal protein
March 10 and 11	21	0	0.16	0.072	After protein restriction
March 12 to 14	30	0.04	0.20	0.098	After continued protein restriction
March 15 and 16	37	0	0.17	0.078	After liberal protein
March 17 and 18	19	0	0.19	0.104	After fasting (March 16 and 17)
March 19 and 20	23	0	0.16	0.060	After liberal protein
March 21 to April 4	120	0	0.18	0.111	Period of moderate protein and moderate total calorie intake
April 6 and 7	23	0	0.13	0.065	After slightly more liberal protein and high total calorie intake
April 22 to May 8	45	0	0.25	0.153	17-day period of rigid protein restriction (8 to 15 grams daily) following 14 days of moderate protein and moderate total calorie intake

* The average time between making the individual aspirations was about 60 minutes before March 8 and about 30 minutes thereafter.

respective periods. A high total calory intake seemed to make the protein more effective in reducing the gastric acidity. Furthermore, fasting (one or two days) appeared to have the same effect as protein restriction.

TABLE 2

Acidity of the fasting gastric contents before, during and after two successive fasts (33 and 41 days respectively)

DATE	NUMBER OT HALF-HOURLY ASPIRATIONS	PER CENT FREE HCl Lowest	PER CENT FREE HCl Highest	PER CENT FREE HCl Average
Before 33-day fast				
1925 May 8	14	0.10	0.25	0.170
9	10	0.05	0.20	0.122
11	12	0.05	0.12	0.085
13	4	0.02	0.12	0.079
15	6	0.05	0.16	0.100
19	9	0	0.14	0.054
20	12	0	0.18	0.054
22	3	0.08	0.13	0.097
25	2	0.08	0.08	0.080
During 33-day fast				
28	21	0.06	0.20	0.133
29	9	0.02	0.19	0.131
30	9	0.07	0.14	0.108
31	5	0.02	0.14	0.074
June 1	16	0.01	0.16	0.076
2	8	0.03	0.15	0.080
3	14	0.02	0.17	0.079
5	15	0.02	0.15	0.093
7	9	0.01	0.15	0.073
9	15	0	0.13	0.056
June 11	13	0	0.17	0.076
12	4	0	0.06	0.030
14	6	0.04	0.13	0.100
16	14	0.04	0.17	0.109
18	4	0.08	0.15	0.105
19	4	0.01	0.17	0.075
21	4	0.08	0.17	0.125
23	7	0.07	0.15	0.110
25	6	0	0.19	0.123
26	2	0.04	0.11	0.075
28	4	0.07	0.19	0.120
29	3	0.08	0.18	0.117
30	5	0.06	0.11	0.084
After 33-day fast and before 41-day fast				
July 2	3	0.13	0.21	0.160
5	5	0	0.29	0.147
7	10	0	0.25	0.128
9	5	0.07	0.22	0.148
10	7	0.08	0.28	0.187
12	6	0	0.25	0.156
July 15	6	0.04	0.22	0.122
16	6	0	0.19	0.068
17	6	0	0.12	0.073
18	5	0	0.18	0.082
19	4	0	0.14	0.076
21	6	0	0.07	0.021
22	4	0.11	0.17	0.133
24	5	0	0.08	0.016
26	4	0	0.07	0.028
28	6	0	0.07	0.030
29	2	0	0	0
30	5	0	0.11	0.028
Aug. 1	7	0	0.09	0.031
2	4	0	0.02	0.005
During 41-day fast				
3	10	0	0	0
4	10	0	0.10	0.032
5	9	0.05	0.14	0.102
6	10	0.02	0.15	0.088
7	7	0	0.18	0.086
8	9	0	0.16	0.089

TABLE 2—*Concluded*

DATE	NUMBER OF HALF-HOURLY ASPIRATIONS	PER CENT FREE HCl		
		Lowest	Highest	Average
Aug.				
9	5	0.01	0.13	0.077
10	8	0	0.15	0.068
11	7	0.02	0.16	0.087
12	5	0.02	0.13	0.064
13	10	0	0.14	0.060
14	6	0	0.10	0.058
15	7	0.01	0.11	0.057
16	4	0	0.08	0.030
17	7	0.02	0.14	0.053
18	7	0	0.09	0.037
20	8	0	0.13	0.058
22	6	0	0.15	0.075
23	4	0.02	0.07	0.045
25	8	0.05	0.17	0.089
29	6	0.02	0.11	0.067
30	3	0	0.04	0.020
Sept.				
1	8	0	0.10	0.058
3	7	0	0.08	0.062

DATE	NUMBER OF HALF-HOURLY ASPIRATIONS	PER CENT FREE HCl		
		Lowest	Highest	Average
Sept.				
6	3	0	0.11	0.067
7	6	0.03	0.10	0.055
9	7	0	0.09	0.047
10	5	0.03	0.12	0.066
12	6	0	0.11	0.073
13	8	0.02	0.14	0.060
After 41-day fast				
15	4	0.09	0.23	0.167
16	2	0.06	0.22	0.140
17	5	0.04	0.21	0.122
18	3	0.06	0.20	0.123
20	4	0.07	0.16	0.107
22	5	0.09	0.25	0.160
23	7	0.07	0.22	0.129
25	5	0.14	0.21	0.160
26	2	0.17	0.19	0.180
27	6	0.04	0.18	0.123
28	6	0.14	0.26	0.235
29	7	0.14	0.36	0.256

DATE	NUMBER OF HALF-HOURLY ASPIRATIONS	PER CENT FREE HC		
		Lowest	Highest	Average
Oct.				
1	5	0.08	0.27	0.184
2	6	0.01	0.22	0.130
3	6	0.08	0.17	0.113
4	2	0.12	0.16	0.140
5	7	0	0.21	0.050
6	6	0	0.10	0.055
7	6	0	0.17	0.075
8	6	0	0.06	0.022
9	5	0	0.01	0.002
10	6	0	0.07	0.023
11	3	0	0.01	0.006
12	5	0	0.05	0.026
13	5	0	0.11	0.040
14	4	0	0.04	0.010
15	5	0.05	0.16	0.112
16	4	0.01	0.18	0.090
17	6	0	0.12	0.073
18	4	0	0.12	0.065
19	6	0.06	0.16	0.108
20	5	0.03	0.17	0.096
21	10	0	0.21	0.113

In addition to this, it was found that the average acidity also fluctuated somewhat from day to day independent of the protein intake. However, these fluctuations were either small, transitory or explainable as being due to imperfections in the technique. For instance, it is clear from the range of acidity found under all circumstances (lowest and highest values in tables 1 and 2) that reliable data might not have been secured on days when only a few aspirations were made. Nevertheless, some fluctuations in acidity independent of the protein intake must also be granted.

Prolonged fasting. Inasmuch as the gastric acidity seemed to rise in the short experiment with fasting which is referred to in table 1, it was thought that a greater rise would result in more prolonged fasting. This

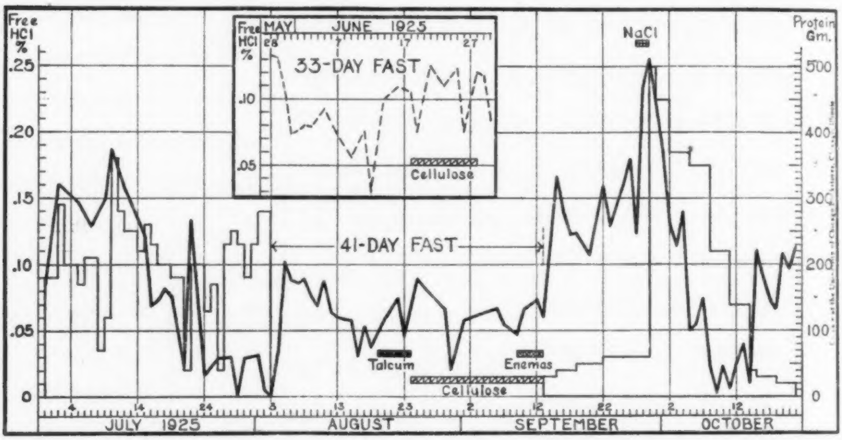

Fig. 1. Acidity of the fasting gastric contents before, during and after 41-day fast (*continuous line*). Columns = approximate protein intake. NaCl = about 20 grams added to the diet each day (2 days); none added during 14 days before or afterward. Inset (same scale) = acidity of gastric contents during 33-day fast.

expectation was further supported by the findings in an earlier study of gastric function on the same subject (Carlson, 1918). It then appeared that the acidity of the gastric contents increased somewhat during a 15-day fast. It was therefore a matter of some surprise to find that the acidity decreased during a 33-day fast (table 2 and fig. 1). In saying that the acidity decreased during this fast, the average acidity found in making 21 half-hourly aspirations on the first day is considered to be the control.

After this fast, the acidity rose above the level at which the fast began. This confirms the earlier results (Carlson, 1918) as well as the findings of Miss Kunde (1924). Nevertheless the acidity after this fast did not rise much higher than it was at the close of a period of protein restriction which

ended May 8 (tables 1 and 2). The failure of the acidity to rise higher was attributed to the acid-reducing effect of a high protein diet which was used immediately after the fast (June 30 to August 3, fig. 1). The effect of variations in the protein intake during this period was also evident. However, it seems that it took larger amounts of protein to reduce the acidity after the fast than before and protein restriction was also followed by a sharper rise.

A second fast (of 41 days) was begun 34 days after the 33-day fast ended. One object was to determine whether a higher gastric acidity would be reached if protein were restricted immediately after fasting. When the 41-day fast began, no free acid was found in the stomach at any time during the first day (table 2 and fig. 1). The high protein diet had so reduced the HCl production that even the combined acid was lower than usual. In this connection, it was of interest to find that the combined acid varied, within narrow limits, in relation to the periodic gastric motility just as the free acid had been found to vary (Hoelzel, 1925). With such a beginning, the acidity rose for two days until it reached a level approximately equal to that of the 33-day fast at a similar stage. The acidity then decreased in both fasts until about the 15th day. After that, it rose somewhat in both fasts but it did not rise as much in the second fast as in the first. The cause of this rise in acidity about the 15th day is not clear but it may be associated with other changes in the fasting organism which led Morgulis (1923) to divide starvation into four phases—of about 15 days each in man.

As indicated in figure 1, non-nutritive substances (flavored with saccharin) were ingested during the latter part of both fasts. Cellulose (370 grams Cellu flour mixed with 510 grams petroleum jelly) was used between the 22nd and 31st days of the 33-day fast. During the 41-day fast, a mixture of 570 grams talcum powder and 170 grams petroleum jelly was used between the 17th and 21st days and a mixture of 880 grams Cellu flour with 570 grams petroleum jelly was used during the remainder of the fast. One aim in using these substances was to find out whether the gastric acidity would thereby be altered. In the earlier study of fasting gastric function by Carlson no rise in acidity had been found in an 8-day fast during which cotton fiber was ingested. As may be seen by figure 1, the acidity rose in the 33-day fast before cellulose was taken and it did not rise as much in the 41-day fast when non-nutritive material was taken earlier in, and longer during, the fast. It therefore seems that the rise in acidity after the 15th day in both fasts was largely independent of the materials taken. It should also be borne in mind that 12 hours or more usually elapsed between the time when these substances were taken (the preceding evening) and the period when aspirations were made. Nevertheless, the use of talcum and cellulose may have led to a slightly higher gastric acidity

because there was less duodenal regurgitation with their use than when water alone was used. Duodenal regurgitation increased about threefold after the first few days of fasting. Increased regurgitation during fasting had also been noted in Carlson's study (1918).

Besides cellulose, enemas were tried during the last four days of the 41-day fast. They did not appear to cause any definite change in gastric acidity.

As indicated in figure 1, the average acidity fluctuated more or less from day to day independent of the main trend of the curve during fasting. This proves that some factor or factors other than diet or the state of nutrition also affect the gastric acidity. Moreover, the fluctuations became greater as the fasts progressed. This probably testifies to an increasing instability of the organism which also seemed to be reflected by corresponding variations in subjective sensations.

It was thought that protein restriction after the 41-day fast would result in a progressively higher gastric acidity. But no evidence of any tendency in such a direction was obtained during the first 14 days after fasting (September 13 to 27, fig. 1). This can not be explained by an insufficient degree of protein restriction. The protein during this period was derived mainly from increasing quantities of fruit and vegetables. It was probably not very well utilized as it appeared to pass through the digestive tract too rapidly. The amount of protein, although not as small as during most other periods of protein restriction, bore no adequate relation to the needs under the circumstances nor to the fat and carbohydrate intake. It became clear later that a higher acidity might have been reached at this time if a more liberal quantity and better quality of protein had been used. The data obtained in the earlier experimenting (table 1) already indicated this possibility but this was not seen until later.

The development of edema, at this time, complicated the situation. Edema had been noted after the 33-day and other fasts but it was usually of a more transient character. As some edema was expected, NaCl was restricted. It hardly seems that this restriction alone could have created a chloride deficiency sufficient to check the HCl production. But, as the conclusions of Lim and Ni (1926) suggest, the edema may have led to a retention of the available chloride in the tissues and thus less may have been available for the production of HCl. This seems to be indicated by the fact that a marked rise in acidity took place after NaCl (about 20 grams each day) was added to the diet on the last two days of protein restriction (September 28 and 29, fig. 1). With this salt intake hydration did not increase at a faster rate (judging from the subject's weight) than before salt was used. Hence there was evidently more chloride available for the production of HCl. On the other hand, no similar rise took place in a later period of protein restriction when similar quantities of salt were added

to the diet on three successive days (December 1, 2 and 3, fig. 2). A small rise, which however may not have been due to the salt, took place on the first day of the later experiment. Diuresis began within 24 hours and the acidity fell on the following two days in spite of the continued excessive salt intake. As edema was absent during this period, its presence after the 41-day fast seems to account for the different results. The period of protein restriction after the 41-day fast was brought to a close because it was believed to be largely responsible for the edema and because it kept the subject feeling dissatisfied and "somewhat starved" in spite of a large total calory intake.

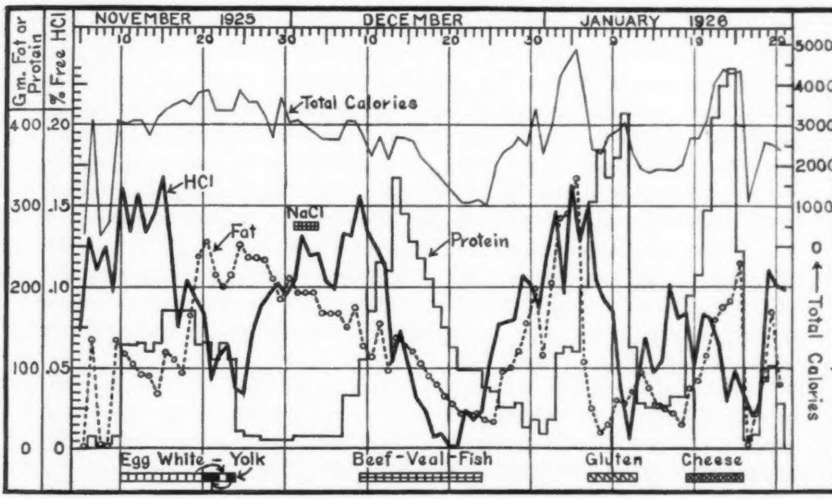

Fig. 2. Showing changes in the acidity of the fasting gastric contents and variations in the protein, fat and total calory intakes. NaCl = about 20 grams added to the diet each day (3 days); none added before or during 7 days afterward; then 2 to 5 grams used daily. The sources of from 85 to 95 per cent of the protein for the corresponding periods indicated below.

The period of protein realimentation which followed (September 30 to October 9, fig. 1) involved the largest protein intake for any period of similar length during this study. The consequence was a sharp decrease in gastric acidity. The enormous protein intake (chiefly meat) was quite fully appreciated and led to a considerable improvement in the subjective sensations during the first few days. It was continued after that mainly in the hope of improving the edema which appeared to be arrested immediately. However, when the gastric acidity became low, the appreciation of protein decreased, gastric regurgitation and a sense of depression

developed and the protein intake therefore had to be reduced. In other words, the available HCl seemed to determine the protein tolerance. With more complete protein restriction (October 15, ff., fig. 1) the acidity rose immediately. But the initial rise was followed by a temporary fall in acidity during continued protein restriction. Evidence of this phenomenon was seen repeatedly during this study. It seemed to parallel the fall in gastric acidity following the initial peak in prolonged fasting.

The effect of protein other than meat. The preceding experimentation with variations in the protein intake referred mainly to differences in the amount of meat (including fish) ingested. It might have been concluded that the results were peculiar to the use of meat or to something other than protein in meat. Hence, at the suggestion of Doctor Carlson, egg white was tried. The use of liberal amounts during three successive days failed to reduce the gastric acidity. Egg yolk was therefore tried. This definitely decreased the acidity. In a subsequent brief experiment, egg white again failed to reduce the acidity. The whites were used after being coagulated in boiling tomato juice or in a double boiler. It seemed difficult to ingest larger quantities as the whites of from 29 to 48 eggs were used each day during the aforesaid trials. However, it seemed possible that the subject had not entirely recuperated from the effects of the 41-day fast plus the subsequent protein restriction and that more thorough protein realimentation might have been necessary before egg white would reduce the gastric acidity. A more prolonged experiment with egg protein was therefore undertaken.

The results are indicated in figure 2 (November 10, ff.). The use of the whites of from 30 to 32 eggs on five successive days failed to reduce the gastric acidity. With the use of 42 coagulated egg whites, the acidity decreased. When 42 egg whites were used raw (November 17), the acidity increased. Egg yolk was also tried again to compare its effect with the effect of egg white to which enough fat (sweet butter) had been added to make it equal in fat content to egg yolk. Egg yolk nevertheless was found to be more effective in reducing the gastric acidity than a proportional quantity of egg white plus butter.

Besides egg white and yolk, vegetable protein (gluten) and casein were found to have an effect similar to meat (fig. 2, December 26, ff.). In brief trials, it did not seem possible to obtain satisfactory results by using nuts or beans as a source of vegetable protein. Nuts (ground almonds and peanut butter) appeared to contain too much fat or oil to make it possible to obtain a sufficiently high protein diet without occasioning a greater calory intake than the subject could easily tolerate at this time. Beans caused too much fermentation when used in large amounts. A brand of gluten flour containing over 80 per cent protein[1] proved to be highly effec-

[1] Supplied by Chicago Dietetic Supply House, Chicago.

tive in reducing the gastric acidity. However, it seemed to take a larger amount of this form of protein than of meat, egg white or yolk to produce similar results. Casein, chiefly in the form of a prepared brand of cottage cheese (to which cream had been added), yielded poorer results than the other forms of protein when the quantity ingested is considered. Nevertheless, there can be no doubt that it also tended to depress the acidity. It seemed to act very much like egg white in leaving the stomach rather rapidly—apparently faster than coagulated egg white.

Discussion. *The effect of fat.* Egg yolk, which contains twice as much fat as protein, was found to have one of the most striking effects in reducing the gastric acidity. This raised the question whether the fat ratio in the diet did not account for the results which were attributed to the larger protein intakes. It can be definitely stated that this is not the case although, as will be seen, the fat of egg yolk is a possible exception. The highest fat intake occurred when the highest gastric acidity was reached during the period of protein restriction immediately following the 41-day fast. An average of about 325 grams of sweet butter and 750 cc. of cream, besides olive oil, was used daily during the last half of this period. Aside from this particular period, practically all other periods of protein restriction involved a relatively high fat intake. This was the case because the subject found that, when protein was restricted, a high fat diet was better borne than a high carbohydrate diet. As indicated in figure 2, the gastric acidity rose while the fat intake was deliberately maintained at a high level immediately after the use of egg protein ceased. After this, the acidity decreased during an experiment with meat which involved a decreasing fat intake. Next, the acidity rose with a high fat intake occasioned by the use of nuts and it decreased sharply with a low fat intake when gluten was used. When used in the larger quantities, some of the fat was not digested (greasy or gray stools) but there was no evidence that this undigested fat in the lower part of the digestive tract inhibited the gastric secretion.

The effect of a high calory intake. In most instances, the ingestion of the larger quantities of protein involved a high calory intake. Might not the results which were attributed to the high protein intakes have been more directly due to the high calory intakes? Some observations presented in table 1 and the results with the use of egg protein seemed to indicate that a high total calory intake considerably enhanced the effect of the protein. On the other hand, some periods of protein restriction which included a large fat intake, and consequently involved a high total calory intake, had the opposite effect of the periods which included a liberal protein intake. To shed more light on the question regarding the possible necessity of a high calory plus a high protein intake to reduce the gastric acidity an experiment was made with the use of lean meat (December 7,

ff., fig. 2). The results proved it to be possible to reduce the free acid to practically zero with a moderate or even a subnormal total calory intake! This means that the total calory intake was, at best, a minor factor in determining the results.

The effect of appetite. The experimentation in this study was partly based upon, and further supports, the conclusion of Carlson (1916) that there is normally, in the fasting stomach of man, a continuous or spontaneous secretion which is not an appetite secretion. However, those who accept the contention of Pavlov (1910) that a normal acid secretion of the fasting stomach is an appetite secretion may regard the results of the variations in the protein intake to have been the consequence of the effect of the diet upon the appetite. That is, they may hold that the secretion of acid juice was inhibited by sating or cloying the appetite with protein and that it was stimulated when a need of, or craving for, protein developed in consequence of its restriction.

While this explanation very likely applies in some degree, the writer does not consider appetite to have been a large factor in determining the acidity of the fasting secretion during this study. In order to determine what allowance should be made for the appetite factor, an attempt was made to find out to what extent the subject was influenced by the contemplation of eating. Observations made during periods when the subject was not eager to eat or was distracted from thoughts of eating were considered to be reliable controls. Some tests made while the subject was preparing food and eager to eat showed a distinct response (appetite secretion) but never a great one. Other tests, when the subject seemed to be equally eager to eat, showed no response and, not infrequently, the gastric acidity decreased. The decreases were due to the usual fluctuations in the acidity of the fasting gastric contents which the writer previously reported. The degree of appetite response therefore bore no clear relation to the general desire for food. However, it appeared to be more closely related to the desire for protein than to the desire for other food. It was impossible to induce a secretion of free acid when none was secreted spontaneously. On the other hand, it was difficult to avoid a slight appetite response near the usual time of eating when the spontaneous gastric acidity was already high. A small, but nevertheless distinct, appetite response was elicited at the end of both fasts although the control acidity was relatively low. Here the psychic factor was evidently a greater determinant of the response than the gastric condition. Data obtained by stimulating the appetite were excluded from the accompanying tables and figures.

However, the possible contention that the variations in gastric acidity which were found in this study were due to changes in appetite loses its force entirely by the fact that evidence of corresponding variations in the

digestive secretion was obtained. No systematic study of the digestive secretion was undertaken but determinations of the acidity of digestive fractions were made from time to time. Thus, no free acid was found in three fractions obtained during the last three hours that food still remained in the stomach before the 41-day fast began. There was therefore not only no appreciable appetite secretion but also no appreciable response to chemical or mechanical stimuli. On the other hand, the highest acidity during digestion was noted when the fasting or interdigestive acidity was highest. In short, these findings support the opinion that the effects on the fasting secretion can be accepted as an index of the effects on the digestive secretion. That a relation between the two exists in most instances has been indicated before—notably by the work of Diehl (1923)—but the observations made in this study help to prove that this case was not an exception.

The more direct cause of the variations in gastric acidity. When the acid-reducing effect of the larger protein intakes was first noted, it was assumed that the activity of the acid secreting mechanism was in some way depressed by the excess of protein after it was digested and absorbed. This explanation, although possibly true to some extent, could not very well account for the differences in the effects of the various sources of protein which were used. It seems that these differences can be better explained by attributing the results mainly to the direct effect of the various foods on the gastric secretory mechanism during digestion. Meat, for instance, is known to be one of the best stimulants of the acid secretion. Raw egg white is known to leave the stomach rapidly without exciting the acid secretion very much. Coagulated egg white is a better gastric stimulant but it does not compare well with egg yolk. Babkin (1914) explains the marked acid secretion induced by egg yolk as being due to stimulation by the digestion products of the yolk fat. London (1925) states that as much as 30 per cent of yolk fat may be digested in the stomach. Gluten very likely is a somewhat weak gastric stimulant and was effective chiefly because it furnished much protein in proportion to bulk. The cottage cheese was probably less effective in stimulating the gastric secretion than it otherwise might have been because of the added cream and the apparent speed with which it left the stomach. The various foods therefore seem to have reduced the gastric acidity in proportion to their power to stimulate the acid secretion. The conclusion consequently seems warranted that the larger protein intakes decreased the acidity by more or less fatiguing the secreting mechanism. Conversely, protein restriction seems to have increased the acidity mainly as a result of functional rest. In other words, a limited (but modifiable) secretory capacity or potential is indicated.

This may raise the question whether the results in this study were not due to gastric secretory limitations peculiar to the subject. The opinion

among physiologists seems to have been that, as Lim and Ni (1926) expressed it, "the stomach is not fatigable under physiological conditions." But this conclusion has evidently been based mainly upon observations on dogs. It may apply to carnivores in general as their gastric mechanism would be expected to be well adapted to a high protein intake. Nevertheless it seems possible that even a carnivore like the dog may have the power to secrete acid juice somewhat reduced as a consequence of a sufficiently high protein intake. May not this explain the conclusion of Pavlov that the well-fed animal does not spontaneously secrete an acid juice between the digestive periods? However this may be, a greater fatigability of the stomach or a more limited gastric secretory capacity on the part of non-carnivorous animals, including man, is to be expected. At any rate, the gastric secretory capacity of the subject of this study can not be regarded as having been subnormal since far larger quantities of protein were required to reduce the acidity below what may be regarded as normal than what would be sufficient for maintenance.

However, fatigability of the gastric secretory mechanism by protein or other gastric secretory stimulants will not explain the tendency of the acid secretion to decrease, after the initial peak, in prolonged fasting. This decrease and the repeatedly observed temporary decreases which followed the initial increase during periods of protein restriction probably reflect the consequences of starvation or protein starvation upon the organism as a whole. For instance, the stomach was also affected in other ways by starvation. The rate of secretion on the 35th day of fasting appeared to have decreased to about one-third of the rate before fasting. The increased duodenal regurgitation which was observed during fasting was perhaps more directly due to a lowering of tonus at the pylorus than to increased reverse peristalsis. Increased duodenal regurgitation (not explainable by higher acidity) was also noted in some instances after simple protein restriction. Sufficiently prolonged protein restriction involving some degree of protein starvation would probably lead to a decrease in gastric acidity by impairing nutrition. Conversely, the fact that a moderate protein intake after a period of restriction (or fasting) usually led to a higher acidity than that attained by protein restriction alone seems to be explainable by the effect of the protein in improving the general nutrition.

Clinical application of findings. The gastro-intestinal disturbances following dietetic excesses may frequently be due to the reduction of the acid secretion by a high protein intake or the use of other strong gastric secretory stimulants. The finding of large quantities of mucus in the stomach at such times may only emphasize the lack of HCl. Observations made during this study make it appear that the mucus is normally digested about as fast as it is secreted but, when the acid secretion is low, the mucus

remains little changed. If, in addition, there is gastric motor quiescence, the mucus accumulates. In such instances, a short fast (which is usually self-imposed by lack of appetite) or protein restriction will be likely to increase the gastric acidity and consequently remove the evidence of a "hypersecretion" of mucus.

However, some instances of hypo-acidity may be due to a subnormal secretory capacity. In such cases, it would appear to be a mistake to attempt to increase the acid secretion by the use of secretins or a high protein intake as this may further overstrain a weak secretory mechanism. A more logical treatment would seem to be HCl therapy with a moderate protein intake or this treatment after some fasting. On the other hand, some individuals may develop hyperacidity because of protein restriction. The "nervousness" to which hyperacidity is frequently attributed may be its consequence rather than its cause. Whether it will be practical to use a high protein diet to reduce the gastric acidity, particularly when ulcer is involved, can only be determined by trial. For such a test, the writer would suggest the use of a diet composed chiefly of lean meat and a protein rich gluten flour. It would seem necessary to force the protein to the point where practically all the free acid is bound by it. Egg yolk would seem to be particularly unsuited for this purpose. Alkali therapy at the end of the digestive periods may be helpful. Necessarily, these are only suggestions given in the light of the findings of this study and given with the knowledge that they are diametrically opposed to current practice.

The writer can be more definite in testifying to the desirability of reducing the acid secretion when the stomach or an adjoining part which can be reached by the acid juice is injured. In the earlier part of this study, it sometimes happened that the gastric mucosa or the mucosa at the cardia bled a little in consequence of the manipulation incident to making repeated aspirations during prolonged periods of observation. This may have been due to applying excessive suction but it is more likely that it was due to pulling on, or withdrawing, the aspiration tube while it was gripped by gastric contractions. On one occasion the tube was tied in a knot. When the acidity was relatively high, considerable pain was occasioned even before evidence of bleeding could be obtained. Moreover, with a high acidity, the pain sometimes continued throughout the digestive period and bleeding was easily induced again on the following day. However, bleeding never occurred with a low acidity and a persistent tendency to bleed easily was cleared up after lowering the acidity with a high protein intake. That the acid or peptic activation caused most of the pain was proven by the soothing effect of removing the acid juice and returning alkaline saliva by way of the tube. A high acidity, plus mechanical irritation by motility, evidently tends to prevent thorough healing.

SUMMARY AND CONCLUSIONS

In a subject who appeared to be unaffected by using moderately large quantities of protein, excessive quantities caused a decrease in the acidity of the fasting gastric secretion. Meat, fish, egg white, egg yolk, vegetable protein (gluten) and casein proved to be effective in reducing the acidity. The decrease is believed to be chiefly due to a fatigue of the gastric secretory mechanism or a limited (but modifiable) secretory capacity. Functional rest, by fasting or protein restriction, was found to raise the gastric acidity again.

It is suggested that the fatigability of the gastric secretory mechanism is a factor in regulating the natural or instinctive protein intake by determining its limits.

During prolonged fasting, the gastric acidity was lower than the control when the control was approximately normal for the subject and it was higher when the control was abnormally low. Immediately after fasting, the acidity was higher than immediately before fasting. Minor fluctuations in the fasting gastric acidity, which are independent of the diet or the state of nutrition of the organism, were noted.

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ADRENAL SECRETION DURING ANAPHYLACTIC SHOCK

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Smith and Ravitz (1920) found no alteration in the epinephrin content of the adrenals in animals killed by anaphylactic shock. These experiments give no insight into the state of adrenal secretion during anaphylactic shock. We have thought it of interest to study the problem by means of Tournade and Chabrol's transfusion method, which permits a direct estimation of any increase in adrenal secretion. Two different conditions were produced; first, a local shock within the tissue of the gland, and second, a general anaphylactic shock.

The animals were anesthetized with chloralose, both vagi cut and artificial respiration given. Blood-pressure curves were recorded by means of mercury manometers and the heart rate of the recipient was registered. This animal's heart had previously been denervated by the removal of both stellate ganglia.

Local anaphylaxis: Injection of 0.1 or 0.15 cc. horse serum into the adrenal gland does not increase the epinephrin output. In six experiments no rise of blood pressure or increase in heart rate was observed in the recipient after the injection had been made into the donor's adrenal.

In another series of experiments dogs were sensitized by subcutaneous injections of 2.5, 5 and 10 cc. normal horse serum, with an interval of 4 days between injections. After an interval of 20 days they were anesthetized and the left lumbo-adrenal vein was anastomosed with the jugular of a non-sensitized recipient. Horse serum (0.1 to 0.15 cc.) was then injected into the donor's left adrenal. In one experiment no increase was

TABLE 1

DATE, 1925	WEIGHT		DOSE OF SERUM	VARIATION IN BLOOD PRESSURE		INCREASE IN HEART RATE OF RECIPIENT	DURATION OF DISCHARGE
	Donor	Recipient		Donor	Recipient		
	kgm.	*kgm.*	*cc.*	*mm. Hg*	*mm. Hg*	*beats per min.*	
8.19	17	8.5	0.15	0	14	0	20 sec.
10.17	24	15	0.1	0	43	12	4 min.
10.20	36	14	0.1	0	22	4	20 min.
10.22	31	10	0.1	0	14	2	4 min.
10.23	20	8	0.1	0	0	0	

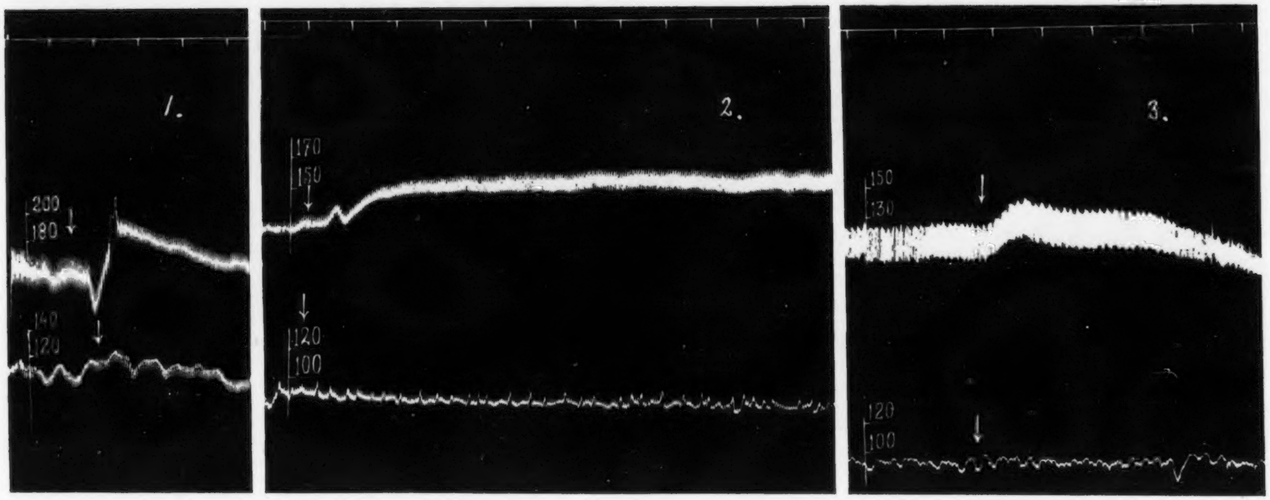

Fig 1. 10/17/1925. Time in minutes. Blood-pressure curve of recipient; blood-pressure curve of donor (sensitized); 0.1 cc. normal horse serum injected into the donor's left adrenal gland.

Fig. 2. 10/20/1925. Time in minutes; blood pressure of recipient; blood pressure of donor (sensitized); 0.1 cc. normal horse serum injected into the donor's left adrenal.

Fig. 3. 10/22/1925. Time in minutes. Blood pressure of recipient; blood pressure of donor (sensitized); 0.1 cc. normal horse serum injected into the donor's left adrenal.

observed, but in four cases an evident discharge was produced. The amount secreted was less considerable than that observed under other experimental conditions. Its effects corresponded with those of an injection of 0.005 mgm. epinephrin, but they were generally prolonged; 4 minutes in two cases and 20 minutes in another.

Anaphylactic shock: The dogs were sensitized as in the experiments just referred to. The shock was produced by injecting from 1 to 4 cc. horse serum into the jugular of the donor. The blood pressure fell from 45 to 78 mm. Hg. The blood pressure of the recipients showed no alteration for from 5 to 8 minutes; then, in four cases out of five, a slight rise was observed and in two cases this was accompanied by an increase in the heart rate. The slight discharge appeared while the donor's blood pressure was low in one case and while it was rising in the other three experiments. The possibility exists that the increased output was due to circulatory changes but, as it continues for some time and increases progressively, we believe it to be a true increase in secretion. It should also be noted that it generally occurs while the blood-pressure level is returning to normal.

TABLE 2

DATE, 1925	WEIGHT		DOSE OF SERUM	VARIATION IN BLOOD PRESSURE		INCREASE IN HEART RATE OF RECIPIENT	TIME BETWEEN INJECTION AND APPEARANCE OF DISCHARGE
	Donor	Recipient		Donor	Recipient		
	kgm.	*kgm.*	*cc.*	*mm. Hg*	*mm. Hg*	*beats per min.*	*min.*
8.19	17	8.5	4	70	10	10	6
10.17	24	15	2.5	45	12	14	6
10.20	36	14	4	70	13	4	5
10.22	31	10	1	50	8	0	8
10.23	20	8	1	78	0	0	0

Controls showed that a single injection of normal horse serum did not increase the epinephrin output.

SUMMARY

Injection of normal horse serum into the adrenal glands does not increase the epinephrin output.

In sensitized dogs an injection of normal horse serum into the gland produces a moderate discharge that continues for a few minutes.

During anaphylactic shock a slight discharge is produced, generally appearing when the blood pressure starts to rise.

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ADRENAL DISCHARGE PRODUCED BY DRUGS INJECTED INTO THE ADRENAL

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When studying the effects of nicotin on adrenal secretion it was found that the injection of this drug into the adrenal gland produced a marked increase of the epinephrin secretion. In this paper the effects of several other drugs have been studied by the same method.

PREVIOUS WORK. In the literature no reference has been found on the effects produced by injecting drugs into the adrenal tissue. Perfusion experiments, a technique closely related to direct injection into the tissue, have been made repeatedly to study adrenal secretion or the vascular reactions of the gland. Masuda (1921) perfused the rabbit's adrenal with normal saline. Schkawera and Kusnetzow (1923) used bovine adrenals maintained at 38°C. and perfused the arteries with Locke's solution. Kudrjazew (1924) and Nikolaeff (1924) worked with the same method. Takenaga (1924) used Tyrode's fluid in perfusion experiments on the dog's adrenal.

The vascular reactions of the adrenals have been studied in detail. The adrenal veins have a thick muscular coat, their structure, innervation, and their physiological and pathological reactions have been described by Peindarie (1920), Dubreuil (1920), Maresch (1921), Sheppard (1922) and Kashiwagi (1922).

Adrenalin injected into the circulation reduces the volume of the dog's adrenal, as it does that of other abdominal viscera (plethysmographic method in the intact animal, Hallion, 1920). Perfusion experiments with Tyrode's fluid showed that a concentration of 1:10,000,000 adrenalin constricted the dog's adrenal (Takenaga, 1924). The volume of the rabbit's adrenal diminishes when perfused with an adrenalin solution of 1:10,000, in normal saline, in some cases a concentration of 1:50,000 was effective (Masuda, 1921). The bovine surviving adrenal does not react in this way to the presence of adrenalin in the perfusing fluid; sometimes only a very slight constriction was observed, at others a dilatation was recorded (Schkawera and Kusnetzow, 1923). Wertheimer (1922) while studying the capillaries of the frog's adrenal *in situ* saw them dilate when adrenalin was injected into the circulation.

Concentrated salt and concentrated hydrochloric acid solutions dilate the vessels of the gland; dilute solutions of the same substances produce constriction. Alkaline solutions and cholin also produce constriction. Cocain, chloral hydrate and amyl nitrite are vasodilators. These results were obtained by perfusing the rabbit's surviving adrenal (Masuda).

The blood vessels of the bovine surviving adrenal are less sensitive to vasoconstrictors (adrenalin, histamin, barium chloride, strophanthin and nicotin) than are other vascular regions of the same animal. Conversely, they dilate more easily when caffein is perfused (Schkawera and Kusnetzow).

Nikolaeff (1924) found that phenol and coniin are weak vasoconstrictors; pyrocatechin and tyramin also constrict but produce a secondary dilatation. Narcotics of the fatty series are vasodilators; alcohols have a dilating effect in direct proportion to their molecular weight; casein is a dilator; peptone has only a very slight dilating power. Tyrosin and its peptids have no effect on the adrenal's blood vessels.

The vessels of a dog's surviving adrenal, perfused with Tyrode's fluid, constrict when epinephrin, histamin or cholin are added even in concentrations of 1:10,000,000 (Takenaga, 1924).

Krichel, quoted by Eichholtz (1923), found no change in the adrenal veins in the presence of nicotin, atropin, strychnin, adrenalin and the bases of quaternary ammonias.

Locke's fluid is a vasoconstrictor after it has passed through the adrenal (Schkawera and Kusnetzow, 1923; Kudrjazew, 1924). This is not due to the presence of epinephrin extracted from the gland, as this property persists after heating, treatment with alkali, and prolonged standing. The pink color given by epinephrin is not present. If epinephrin is added to the fluid, the tests mentioned above cause it to deteriorate so that the presence of protective substances can not be invoked to explain a possible persistence of adrenalin. The constricting power of the fluid is equivalent to that of an adrenalin solution of from 1:20,000 to 1:100,000. In the cortex a muscarin-like substance was found. The constricting substance is more active at 38° to 40°C. than at 9°C. It is more abundant in the anterior part of the gland, and is not extracted by washing out but is secreted into the fluid, because it can be obtained for several days. By this method, moreover, a greater quantity of epinephrin is obtained than by simple extraction. Nicotin increases this secretion 400 to 600 per cent with no preliminary diminution. Eserin also increases the secretion, but not so intensely (150 per cent); pilocarpin does not affect it. Schkawera and Ssentjurin (1925) found that fluid which had perfused the testicle increased the secretion. Chloroform and chloral hydrate have no effect in dilute solutions, but in concentrations of from 1:200 to 1:500 they first increase and then diminish the secretion. Peptone, casein, ethyl alcohol

(0.1 per cent), amyl alcohol, acetone, nicotin and tyramin increase the secretion; tyrosin is inactive but one of its peptids increases it slightly; methyl alcohol diminishes the secretion. Tyramin does not alter the chromaphil reaction of the adrenal medulla.

The perfusion of the isolated bovine adrenal with concentrated solutions of sodium carbonate (1:10,000 or more) increases the epinephrin secretion. More dilute solutions (1:100,000) are less effective. Concentrated solutions of lactic acid increase the secretion. The maximum is obtained when the pH of the perfusion fluid is 7.32. Concentrations of 1:3,000 lactic acid inhibit secretion; lower concentrations (1:4,000) are inactive.

Experiments. Dogs anesthetized with chloralose (0.10 gm. per kgm. given intravenously) were used in all the experiments. Tournade and Chabrol's transfusion technique was followed. The donor's blood pressure, the recipient's blood pressure, and the rate of the denervated heart were recorded.

The donor's left adrenal was well separated from the surrounding tissues along its external border. A forceps holding the connective tissue near the gland served to maintain it fixed while a fine needle was inserted in its external border and directed towards the center. The fluid (generally 0.1 cc., exceptionally 0.2 cc.) was then injected from a very exactly calibrated syringe. The needle was rapidly taken out.

The discharge obtained was more marked when the injection was made either immediately above or below the lumbo-adrenal vein. No injections were made below a point 3 mm. distant from the lower extremity of the gland. It is inadvisable to make more than four or five injections in one gland since this adds to the sources of error. One such source that has been observed is the discharge produced by an inactive substance when injected in the same place previously used for the injection of an active drug. The amount discharged is generally the same in two consecutive injections of equal doses of a drug. Sometimes, however, different reactions are obtained under these conditions, due, principally, to the different locations of the two injections; e.g., one being made near the center, the other near the inferior extremity of the gland. The latter produces a smaller discharge. As is to be expected, all animals are not equally sensitive and their reactions vary accordingly.

Stewart (1912), Popielski (1913), Stewart, Rogoff and Gibson (1916) and Gautrelet (1923) have proved that massage of the adrenal produces an epinephrin discharge, a fact that we have confirmed. Our results might, therefore, be due to the handling of the gland and not to the action of the drugs. This argument may be disregarded because we have repeatedly observed that when care is taken in handling the gland and in introducing the needle, the epinephrin discharge is not sufficient to modify the blood pressure and the rate of the denervated heart of the recipient. Control

TABLE 1

DATE, 1925	WEIGHT		DRUG INJECTED	AMOUNT	RISE IN BLOOD PRESSURE		INCREASE IN HEART RATE OF RECIPIENT
	Donor	Recipient			Donor	Recipient	
	kgm.	*kgm.*			*mm. Hg*	*mm. Hg*	*beats per min.*
7/20	23.5	11.0	Nicotin	0.00001 mgm.	0	0	0
7/20	23.5	11.0	Nicotin	0.0001 mgm.	0	52	40
6/5	23.0	9.3	Nicotin	0.15 mgm.	0	90	64
*9/15	21.5	14.0	Nicotin	0.05 mgm.	0	62	44
6/30	31.0	29.0	Eserin	0.0002 mgm.	0	0	4
6/30	31.0	29.0	Eserin	0.002 mgm.	0	110	22
6/20	30.0	9.5	Eserin	0.01 mgm.	0	130	36
6/17	30.0	11.0	Eserin	0.1 mgm.	0	60	48
*9/15	21.5	14.0	Eserin	0.01 mgm.	0	0	0
*9/15	21.5	14.0	Eserin	0.1 mgm.	0	0	0
7/28	29.5	10.5	Hydrastinin	0.001 mgm.	0	76	30
7/28	29.5	10.5	Hydrastinin	0.1 mgm.	0	74	28
9/8	29.0	13.0	Morphia	0.001 mgm.	0	0	0
9/12	16.0	14.5	Morphia	0.001 mgm.	0	0	0
9/8	29.0	13.0	Morphia	0.01 mgm.	0	80	12
9/12	16.0	14.5	Morphia	0.01 mgm.	0	20	4
7/28	29.5	10.5	Morphia	0.1 mgm.	0	110	72
6/30	31.0	29.0	Adrenalin	0.025 mgm.	0	0	2
7/20	23.5	11.0	Adrenalin	0.025 mgm.	0	74	58
7/21	19.5	11.5	Adrenalin	0.025 mgm.	0	94	78
6/20	30.0	9.5	Arecholin	0.01 mgm.	0	0	0
6/19	30.0	9.5	Arecholin	0.05 mgm.	0	0	0
6/17	30.0	11.0	Arecholin	0.1 mgm.	0	68	22
7/21	19.5	11.5	Acetylcholin	0.001 mgm.	0	0	0
7/21	19.5	11.5	Acetylcholin	0.1 mgm.	0	80	82
7/28	29.5	10.5	Quinin	0.1 mgm.	0	48	0
*7/25	19.0	11.5	Quinin	0.1 mgm.	0	0	0
*7/25	19.0	11.5	Quinin	0.01 mgm.	0	0	0
*7/24	17.5	15.5	Atropin	0.001 mgm.	0	21	6
*7/24	17.5	15.5	Atropin	0.01 mgm.	0	0	0
*7/24	17.5	15.5	Atropin	0.1 mgm.	0	0	0
*7/24	17.5	15.5	Atropin	0.1 mgm.	0	32	8
9/18	24.0	11.5	Atropin	0.01 mgm.	0	0	0
9/18	24.0	11.5	Atropin	0.1 mgm.	0	0	0
6/17	30.0	11.0	Strychnin	0.1 mgm.	0	28	40
7/28	29.5	10.5	Strychnin	0.1 mgm.	0	10	4
*7/25	19.0	11.5	Strychnin	0.1 mgm.	0	0	0
7/31	17.0	15.5	Peptone	0.1 mgm.	0	10	8
7/31	17.0	15.5	Veratrin	0.1 mgm.	0	14	16
7/28	29.5	10.5	Picrotoxin	0.1 mgm.	0	12	4
7/24	17.5	15.5	Pilocarpin	0.001 mgm.	0	4	4
7/21	19.5	11.5	Pilocarpin	0.01 mgm.	0	22	34
7/24	17.5	15.5	Pilocarpin	0.1 mgm.	0	0	6

TABLE 1—*Concluded*

DATE, 1925	WEIGHT		DRUG INJECTED	AMOUNT	RISE IN BLOOD PRESSURE		INCREASE IN HEART RATE OF RE-CIPIENT
	Donor	Re-cipient			Donor	Re-cipient	
	kgm.	*kgm.*			*mm. Hg*	*mm. Hg*	*beats per min.*
7/24	17.5	15.5	Pilocarpin	0.1 mgm.	0	0	2
7/28	29.5	10.5	L. Alternat.	0.1 mgm.	0	10	4
7/31	17.0	15.5	Cobra venom	0.1 mgm.	0	6	4
7/28	29.5	10.5	Strophanthin	0.1 mgm.	0	10	0
7/25	19.0	11.5	Strophanthin	0.001 mgm.	0	0	0
7/25	19.0	11.5	Strophanthin	0.01 mgm.	0	0	0
7/25	19.0	11.5	Strophanthin	0.1 mgm.	0	0	0
9/8	29.0	13.0	HCl 0.01 N	0.1 cc.	0	70	20
7/24	17.5	15.5	HCl 0.01 N	0.1 cc.	0	0	0
9/12	16.0	14.5	HCl 0.001 N	0.1 cc.	0	0	0
7/24	17.5	15.5	HCl 0.001 N	0.1 cc.	0	0	0
9/8	29.0	13.0	Na(OH) 0.01 N	0.1 cc.	0	70	20
9/12	16.0	14.5	Na(OH) 0.001 N	0.1 cc.	0	0	0
7/31	17.0	15.5	Ergamin	0.1 mgm.	0	0	0
†7/17	30.0	11.0	Scorpion venom	0.1 cc.	0	0	0
†9/18	11.5		Scorpion venom	$\frac{1}{10}$ gl.	0	0	0

Note: The following salts were used: Eserin salicylate, morphin chlorhydrate, quinin chlorhydrate, atropin sulphate, strychnin nitrate, pilocarpin nitrate, histamin phosphate. The other drugs used were bases.

* The left splanchnic was severed.

† One gland in 20 cc. normal saline.

experiments were also performed in which 0.1 cc. and 0.15 cc. of 0.8 per cent sodium chloride solutions were injected in exactly the same way as the drugs. In no case (8 experiments) did we observe a rise of blood pressure or an increase in heart rate in the recipient.

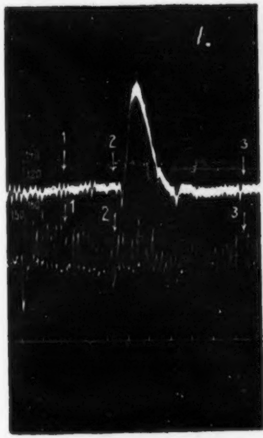
Sometimes when an inactive drug or sodium chloride solution was given, a slight fall was seen in the recipient's blood pressure (fig. 3). This might be due to a small adrenal discharge, but it was never taken into account.

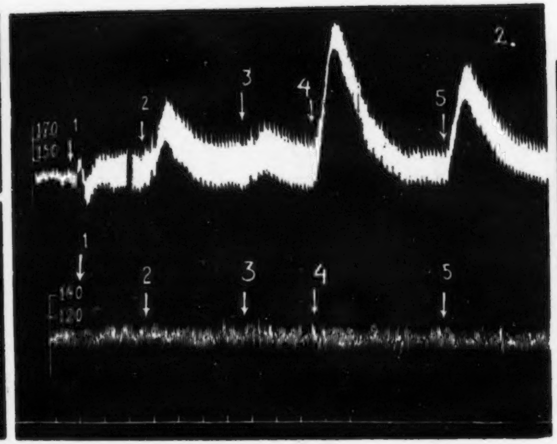
Negative results were also obtained with 0.1 cc. (four experiments) and 0.15 cc. (two experiments) normal horse serum (fig. 1).

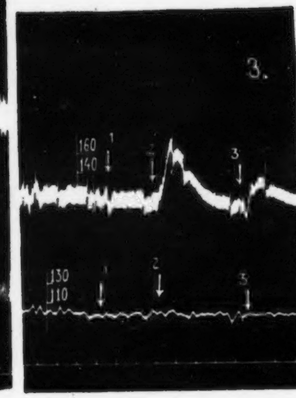
Nicotin is the most potent among the active drugs; 0.0001 mgm. gives a marked discharge, 0.00001 mgm. is not effective. The following drugs also give positive results; eserin (fig. 1), hydrastinin (fig. 2), morphia (fig. 2), pilocarpin (fig. 3) and adrenalin (fig. 4).

These results are somewhat surprising because they disagree with results obtained by intravenous injection of the same substances that will be published later. Intravenously given, eserin produces a discharge, hydrastinin and morphia have an inconstant effect, and adrenalin is inactive.

Other drugs produce a discharge when given in large doses; e.g., 0.1







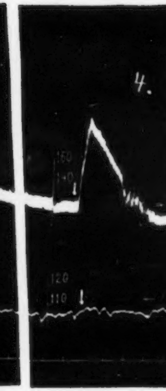


Fig. 1. 6/30/1925. Blood pressure of recipient. Blood pressure of donor. Time in minutes.
1. Injection of 0.1 cc. normal horse serum into the donor's left adrenal.
2. Injection of 0.002 mgm. eserin into the donor's left adrenal.
3. Injection of 0.0002 mgm. eserin into the donor's left adrenal.

Fig. 2. 7/28/1925. Blood pressure of recipient. Blood pressure of donor. Time in minutes.
1. Injection of 0.1 mgm. strophanthin into the donor's left adrenal.
2. Injection of 0.1 mgm. quinin sulphate into the donor's left adrenal.
3. Injection of 0.1 mgm. strychnin nitrate into the donor's left adrenal.
4. Injection of 0.1 mgm. morphine chlorohydrate into the donor's left adrenal.
5. Injection of 0.1 mgm. hydrastinin into the donor's left adrenal.

Fig. 3. 7/21/1925. Blood pressure of recipient. Blood pressure of donor. Time in minutes.
1. Injection of 0.1 cc. normal saline into the donor's left adrenal.
2. Injection of 0.1 mgm. acetylcholin into the donor's left adrenal.
3. Injection of 0.01 mgm. pilocarpin into the donor's left adrenal.

Fig. 4. 7/21/1925. Blood pressure of recipient. Blood pressure of donor. Time in minutes. Injection of 0.025 mgm. adrenalin (0.1 per cent solution) into the donor's left adrenal.

mgm. arecholin, acetylcholin (fig. 3), quinin (fig. 2), atropin and strychnin (fig. 2). The discharge produced by this dose of strychnin is slight and is a contrast to the marked results obtained when it is injected into the circulation where it can influence the central nervous system.

Sodium hydrate and hydrochloric acid solutions are active when given in certain concentration (0.01 N); more dilute solutions (0.001 N) are inactive.

Only slight discharges were obtained with 0.1 mgm. Witte's peptone, strophanthin (fig. 2), veratrin, picrotoxin and the venoms of *Lachesis alternatus* and *Naja tripudians*. Ergamin and the venom of a scorpion (*Butus quinquestriatus*) are inactive.

Sympathico-mimetic (adrenalin, nicotin) and parasympathico-mimetic (nicotin, eserin, arecholin) drugs are equally active. Their effects might be attributed to a local irritative and nonspecific action, but it has been seen that ergamin, veratrin and snake venoms—substances that have a potent local action—have little or no effect when injected directly into the adrenal.

Reflex action can also be disregarded, since positive results were obtained with nicotin and eserin in denervated glands. The amounts discharged were smaller, due possibly to the almost total supression of secretion in denervated glands.

The method is useful in the study of the local action of substances on the gland, but it does not permit any inference as to the action of a drug given intravenously because the effects are not always parallel. In some cases, as with nicotin, the effects are identical; in others, as with strychnin, they are not.

Although at first sight the perfusion of surviving glands appears to be a better method, it produces greater alteration in the normal reactions of the gland; e.g., stimulation of the splanchnic does not greatly increase the adrenal secretion of surviving glands (Kudrjazew, 1924), whereas it increases markedly the secretion of glands perfused *in situ*. Possibly in the surviving gland some alteration may occur in the synapse (splanchnic nerve and medulli-adrenal cell).

SUMMARY

1. Injection into the adrenal gland of 0.1 cc. normal saline solution or normal horse serum does not produce an adrenal discharge.

2. Marked adrenal discharge is produced by the injection of nicotin, eserin, hydrastinin, adrenalin, pilocarpin and morphia.

3. Arecholin, acetylcholin, quinin, atropin and strychnin produce a discharge when injected in large doses.

4. Only slight discharges were produced by Witte's peptone, veratrin, picrotoxin and the venoms of *Lachesis alternatus* and *Naja tripudians*.

5. Negative results were obtained with strophanthin, ergamin and the venom of *Butus quinquestriatus*.

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THE VALUE OF EGG YOLK IN SUPPLEMENTING DIETS DEFICIENT IN CALCIUM

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It has been claimed (McCollum, 1922a) that all diets without milk or leafy vegetables are deficient in calcium and therefore are not suitable for human or animal nutrition.

In a preliminary communication (Tso, 1924) favorable results were reported of the use of egg yolk as a supplement in calcium-poor diets. This paper presents more detailed and additional data to show that egg yolk as a food is possessed of a unique physiological value in supplementing diets deficient in calcium. For example, a diet containing approximately 0.15 per cent of calcium which is generally considered inadequate can be transformed into an adequate ration for the normal nutrition of the experimental animals for four generations by the addition of approximately 5 per cent of fresh egg yolk.

Experimental. Young white rats (Mus norvegicus albinus) were employed as the experimental animals. Four rats were confined in one wire-netting cage without bedding. The cage measures 40 by 20 by 20 cm. and stands 2 cm. above a metallic tray in which were collected practically all of the excreta. The pregnant rat on the experimental diets was removed one to five days prior to parturition and placed in a separate cage containing straw for nesting. After four weeks of lactation the young were weaned and the mother rat was returned to the original cage.

Natural foodstuffs—millet, soybean and dried cabbage—were employed as basic constituents of most of the rations. The millet was the common variety used in North China, namely, *Setaria italica*, and contains 9.2 per cent protein, 76.6 per cent carbohydrate, and 0.2 per cent fat (Hammond and Hsia, 1925). The soybean was the common yellow variety containing, in parts per 100, 39.5 protein, 26.6 carbohydrate, 20.7 fat, 1.7 fiber, 5.2 ash and 6.2 water (Piper and Morse, 1924). The millet and soybean were purchased in the form of freshly ground meal. The cabbage was the common celery-cabbage variety (Brassica campestris B. chinensis) and, on dry basis, had the following percentage composition (Embrey, 1921): protein 25.3, carbohydrate 54.5, fat 1.0, fiber 8.4 and ash 10.7. The cabbage grows in the shape of compact mass 8 to 14 inches

high and 3 to 5 inches in diameter. The dried powdered form used in the experiments was prepared as follows. Each plant was cut into halves, the upper leafy portion was spread out to dry in the sun and the lower portion containing largely the stems was discarded. Final drying was done in an oven at 40 to 50°C. The dried substance was then more or less pulverized in a stone mill.

The egg yolk was separated from the white by pouring the egg back and forth from one portion of the shell into the other. The entire yolk practically freed from the white was emptied into a beaker with the membrane intact. After examination of both the yolk and the white to exclude possible chips of shell matter, they were separately weighed into the food mixtures.

The rations as fed to the rats in series I and II experiments were prepared by mixing the various ingredients with a little more than equal quantity of boiling distilled water and then cooked for twenty minutes in a double boiler. The food was made up twice weekly and fed *ad lib* six times a week. Distilled water was separately given *ad lib.* The animals were weighed weekly.

As determined by McCrudden's gravimetric method (1911), the calcium content of the egg yolk was 0.131 per cent; egg white 0.012 per cent; millet 0.042 per cent; soybean 0.261 per cent; and dried cabbage 0.718 per cent. The phosphorus values of these foodstuffs are quoted from various sources. They are, respectively, 0.45 per cent (Hess, 1923); 0.015 per cent (Forbes and Keith, 1914); 0.334 per cent (Miles and Feng, 1925); 0.82 per cent (Osborne and Mendel, 1917); and 0.48 per cent (Forbes and Keith, 1914).

Series I experiments. Six rats, three and a half weeks old, were fed 6 to 11 months on ration 1 which was made up of 70 parts by weight of millet and 30 parts of egg yolk. This mixture contains 68 mgm. of calcium. Another six rats were put on ration 2 which was composed of 75 parts of millet, 25 of whole milk powder (Merrel-Soule), and 5 of butter. This mixture yields 270 mgm. of calcium. The two diets are more or less comparable in contents of protein, carbohydrate, and fat and in the percentage distribution of the total calories. The rats fed on ration 1 grew to adult size (males, 300 grams and females 180 grams). The female rats all reproduced and successfully weaned their young in most instances. Rat 13 fed on ration 1 for 46 weeks cast three litters of which two were weaned. However, the second generation rats failed to reach adult size. The ration 2 rats grew up vigorously and reproduced young which again grew up at the normal rate. Rat 20 fed on ration 2 for 46 weeks had four litters totalling 38 young all of which were successfully weaned.

Series II experiments. Three litters of young rats were employed. Littermates, as indicated in the charts, were designated by the same letter

A, B or C. Litter A containing 8 young were 29 days old, litter B, 11 young, 30 days old, and litter C, 6 young, 24 days old. Four rats were put on each of five different rations, 10, 11, 12, 13 and 14. Of the four rats on each ration one came from litter A, two from litter B, and one from litter C. Ration 11 was temporarily discontinued on account of the death of one animal in the sixth week of feeding and the evisceration of the carcass by the other animals in the same group. Feeding on this ration was later repeated using 5 rats from three litters.

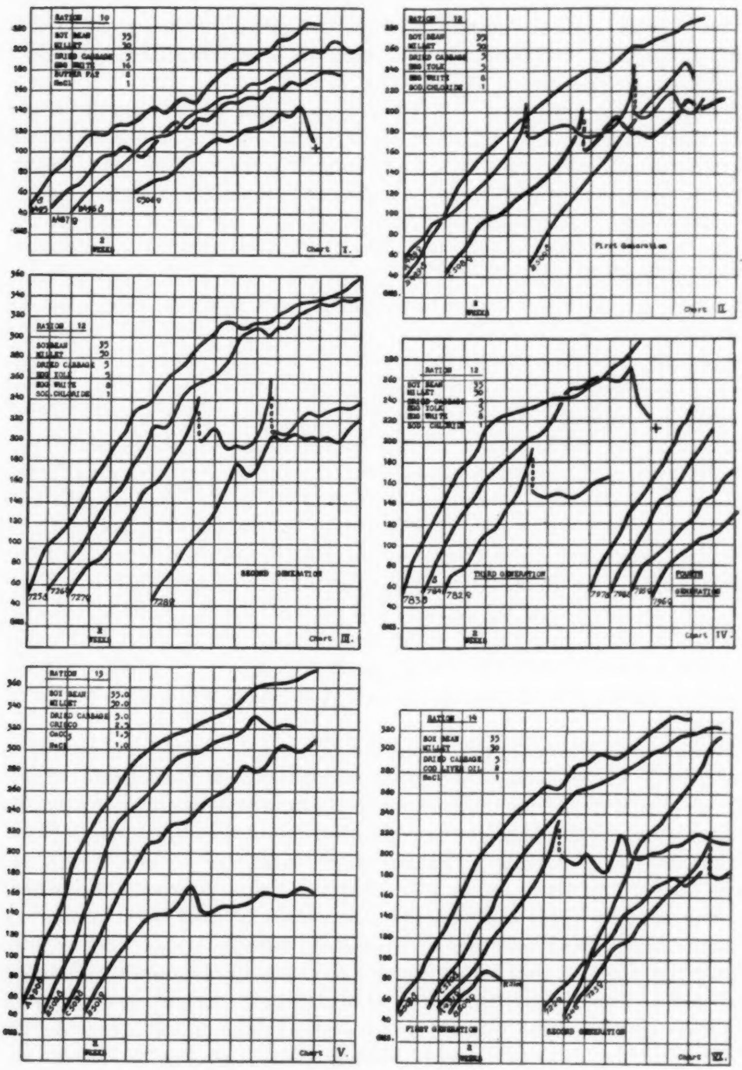
The basic portion of the five rations is composed of 35 parts of soybean, 50 millet, 5 dried cabbage, and 1 sodium chloride. Ration 10 consists of the basic portion supplemented by 16 parts of egg white and 2 parts of butter fat. From chart I it will be seen that on this ration the animals failed to thrive. The animals were stunted and looked ill. On the other hand, 5 parts of egg yolk and 8 parts of egg white added to the same basic portion (ration 12, and charts II, III and IV), transformed the poor basic

TABLE 1

A comparison of the protein, carbohydrate, fat, Ca and P contents and the caloric values of rations used in series II experiments

RATION	TOTAL CALORIES	PROTEIN		CARBOHYDRATE		FAT		Ca	P
		Grams	Per cent of total calories	Grams	Per cent of total calories	Grams	Per cent of total calories	*gram*	*gram*
10	373	21.5	24	44.5	49	11.0	27	0.150	0.480
11	383	21.4	23	44.5	48	12.1	29	0.161	0.523
12	373	22.4	25	44.5	49	10.6	26	0.155	0.501
13	370	19.6	22	44.5	49	11.5	29	0.749	0.478
14	365	19.6	22	44.5	50	11.0	28	0.148	0.478

diet into a highly satisfactory ration on which young rats grew to the normal adult size and reproduced to the fourth generation. The addition of 5 parts of egg yolk to the basic protion of ration 12 introduces about 1.6 gram of fat, 1.3 gram of protein, 0.0065 gram of calcium and 0.022 gram of phosphorus. The calcium represents 4.2 per cent and the phosphorus 4.3 per cent of the total calcium and phosphorus contents of ration 12. Ration 11 differs from ration 12 in having twice as much egg yolk but no egg white. The results are essentially comparable except that the second generation animals grew up even more vigorously. This experiment was terminated much earlier. Ration 13 differs from ration 12 in having 1.5 gram of $CaCO_3$ and 2.5 grams of crisco added to the basic portion. The growth of the rats was excellent (chart V) although there was failure of reproduction. Ration 14 (chart VI) is the basic portion supplemented by 2 parts of cod liver oil. The outcome of this experiment up to its termina-



Charts 1 to 6

tion seems comparable to the results obtained from the 5 per cent egg yolk ration. Table 1 gives a summary of the five rations with respect to contents of protein, carbohydrate, fat, calcium and phosphorus and their percentage representation in the total calories.

SERIES III EXPERIMENTS. "Soybean milk" with the addition of 5 per cent cane sugar and 0.1 per cent sodium chloride was employed as the basic portion of five diets. The soybean milk was prepared like the commercial product except that it was more concentrated and more constant in composition. According to a partial analysis by Doctor Horvath it contained about 4 per cent of protein and 1.6 per cent of fat. On ration 29 which was the basic portion supplemented by 4 per cent of egg yolk, rats grew up normally, reproduced young and these in turn grew up normally. In 22 weeks one female cast one litter and two others two litters each. Of these litters three were successfully weaned. The basic portion supplemented by 2 per cent of soybean oil (ration 30) proved entirely inadequate. The animals ceased to grow after 2 to 4 weeks. Starting at the initial weight of 40 grams the animals gained only 30 to 40 grams during the experimental period of 8 weeks. One rat died in the sixth week of feeding, weighing only 46 grams. Fairly good growth was secured from rations 36 and 42, the former containing 0.15 per cent calcium carbonate and the latter a complete salt mixture (McCollum's 185 salt mixture). In 16 weeks the male rats on both rations gained on the average 210 grams and the females 130 grams. On ration 35 which contained 2 per cent of cod liver oil the male rats grew from 50 to 250 grams and the females, from 46 to 180 grams in a period of 16 weeks. At the age of 20 weeks none of the females except those on ration 29 reproduced young.

DISCUSSION. Sixty-four hundredths per cent of calcium and 0.45 per cent of phosphorus in a diet are considered by McCollum (1922b) as the optimal requirements of growing rats in these elements. According to this standard the phosphorus content of the diets used in series II experiments was adequate. Bethke, Steenbock and Nelson (1923–24) have shown that there exists a certain correlation between the optimal calcium content in the diet and the amount of cod liver oil or rather the vitamin (other than vitamin A) contained therein. When the vitamin supply is optimal (1 or 2 per cent of cod liver oil) the calcium requirement need not be higher than 0.15 per cent of the diet. In the presence of an adequate supply of the vitamin an excessive intake of calcium (over 0.5 per cent of the diet) is not only wasteful but according to Bethke apparently depressing to normal growth and development of the animals. On the other hand, an extreme paucity of this particular vitamin in the food cannot apparently be compensated by a higher intake of calcium salts.

In the case of series II experiments it is clear that the successful outcome

is entirely dependent upon the addition of either cod liver oil, small quantity of egg yolk, or calcium, to the basic portion of the rations which contained approximately 0.15 per cent of calcium. Thus, in what is ordinarily considered calcium-poor diets egg yolk acts like cod liver oil: it furnishes a vitamin-like substance which compensates for the apparent shortage of calcium salts in the diet. The failure of the second generation rats to grow normally on ration 1 which contained an excessive quantity of egg yolk but a total calcium content of 0.068 per cent of the food mixture is apparently due to the fact that the calcium intake fell below the minimum requirement although the vitamin content was more than adequate.

It is interesting to note that leafy vegetables in themselves, such as the Chinese cabbage which was given to furnish 24 per cent of the total calcium in ration 10 have no apparent effect in improving the quality of the diet with respect to calcium deficiency.

The results of these experiments have a practical bearing of fundamental significance on the problem of feeding of young children in such a country as China where cow's milk is little used. To most households the cost of milk seems almost prohibitive. On the other hand, the cost of eggs is extremely low. Therefore, the addition of one or two eggs a day to the diet of Chinese children should be of immense value not only in enhancing its vitamin A and vitamin B content and its protein quality but in improving the calcium metabolism and safeguarding the body against calcium starvation. It will also enrich the diet in blood building elements. It will protect the child against rickets (Hess, 1923; Casparis, Shipley and Kramer, 1923).

SUMMARY AND CONCLUSION

According to McCollum (1922a), the deficiency of calcium in eggs is a serious limitation of their value as a supplementary or protective food and all diets containing no milk or leafy vegetables are poor in calcium and therefore inadequate for normal nutrition. On the contrary, it is now demonstrated that small quantities of egg yolk effectively supplement calcium-poor diets. On a ration containing 0.15 per cent of calcium which barely represents one-fourth of the standard requirement in this element and approximately 5 per cent of fresh egg yolk young albino rats grew normally to adult size and reproduced young to the fourth generation. The same basic ration supplemented by 2 per cent of butter fat was entirely unsatisfactory.

Egg yolk should be considered as efficient a protective food as milk. Whereas milk in relatively large quantities is the most available source of calcium for human nutrition, egg yolk in small amounts furnishes a vitamin like substance which enables the body to mobilize and utilize economically the apparently limited supply of calcium in the diet. Egg yolk is therefore

invaluable in supplementing Chinese diets particularly diets of young children in which milk or milk products take little or no part.

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THE EFFECTS OF RADIATIONS FROM A QUARTZ MERCURY VAPOR ARC UPON SOME PROPERTIES OF PROTEINS[1]

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Before heliotherapy or the study of the effects of artificial radiations upon living matter can be put upon a thoroughly rational basis it seems essential to ascertain more precisely what influence they may exert upon the various constituents of the tissues. One direction of approach leads to the study of the effects on the chief constituent of protoplasm, protein. The greater part of the observations upon this subject hitherto reported have been incident to some other inquiry. Most of them have been confined to blood- and egg-proteins.

In order to ascertain to what extent the changes reported as due to exposure to ultra violet rays have general application, the effects of radiation from the mercury vapor arc upon a variety of proteins, some of them highly purified, have been examined. It has been found that proteins from a diversity of sources show, as the result of such radiation, changes in properties which with one or two important exceptions are similar.

Experimental technique. *Source of radiant energy.* Two quartz mercury vapor arcs of the Cooper Hewitt horizontal Uviarc type were employed. Both arcs were new (1). In the earlier experiments the larger one was used: the current, measured during each exposure, varied from 3.8 to 5.0 amperes and the electromotive force from 150 to 170 volts. The current of the less powerful arc employed at Woods Hole was 3.5 amperes at about 58 volts. The radiated object was kept at 27 cm. from the center of the larger arc, and at 10 cm. from the smaller. In view of the essentially qualitative character of the observations, the possible variations in the intensity of the ultra violet emission have not been taken into account in the present report.

Method of exposure. Fifteen cubic centimeters of the solutions were placed in glass crystallizing dishes 75 mm. in diameter. By using the same amount and containers of the same size the depth of the solution

[1] Part of the expenses of this investigation was met by a grant from The Elizabeth Thompson Science Fund.

was always approximately 3 mm. The contents of each dish were exposed to the radiations either directly, or through a quartz plate 2 mm. thick, or through glass covers averaging 2.7 mm. in thickness. Whenever it was necessary to use a quartz test tube, as in the case of dried material, it was tightly stoppered and sealed with a thick, well dried coat of collodion. By the simple expedient of surrounding the dishes with cracked ice the solutions were always kept well below room temperature during the exposure.

The protein preparations studied. *Ovalbumin* was crystallized according to the Hopkins and Pinkus method (2), dialysed free from sulphates, and evaporated to dryness in a current of air at a temperature not exceeding 40°C. *Lactalbumin,* obtained by Sebelien's method (3), was purified by dialysis and dried at a low temperature. The *hemocyanin*[2] was highly purified and electrolyte-free. *Gliadin, edestin, excelsin,* and *phaseolin*[3] were prepared according to the methods described by Osborne (4). The *other vegetable proteins* were preparations from the laboratory of Dr. D. Breese Jones of the Bureau of Chemistry, Washington.[4]

Most of the samples examined had been kept dry for some time. As a rule, approximately 0.5 per cent solutions were prepared, but in the case of less soluble vegetable proteins, some of which were slightly denatured, this concentration was not always maintained. Water, sodium chloride solution (10 per cent), or alcohol (70 per cent) were used as solvents, depending upon the nature of the proteins involved.

Observational procedure. Comparisons were always made between the non-radiated material and samples of the same solutions which had been exposed to the arc either *a*, directly, *b*, under a quartz plate, or *c*, covered by a watch glass. Inasmuch as no appreciable differences between the non-radiated samples and those exposed under glass were observed, the last procedure was omitted in the later work. It is to be emphasized that for each observation recorded, samples of the same material, radiated and non-radiated, were compared side by side.

Experimental observations. Changes attributable to the effects of radiations and independent of the environmental conditions, were observed in the physical and chemical properties of the following proteins: crystallized ovalbumin, lactalbumin, hemocyanin, edestin, excelsin, phaseolin, arachin, lima bean β globulin, canavalin from the jack bean, flax seed globulin, cantaloupe globulin, and α globulin from the tomato seed.

[2] This material was kindly furnished by Miss Jessie Hendry of the Department of Physical Chemistry, Harvard Medical School.

[3] The phaseolin was prepared by Miss Verz R. Goddard of the Department of Physiological Chemistry, Yale University.

[4] Doctor Jones generously supplied the following proteins for use in this investigation: flaxseed globulin, lima bean β globulin, arachin, canavalin from the Jack bean, cantaloupe seed globulin, tomato α globulin, β globulin from the mung bean, β globulin from the adsuki bean, and cocoanut globulin.

Odor. A characteristic rather unpleasant odor common to all the proteins and resembling that produced when the skin is exposed to an arc for a short time was observed during the irradiation. It was less noticeable when the proteins were irradiated dry, but tended to increase when a solvent was added subsequent to the exposure. It appeared equally readily whether the solution were exposed to the rays directly or under a quartz plate. The purity of the protein cannot be an essential factor since the odor was as evident in ovalbumin recrystallized five times, as in that which had not been reprecipitated at all. The odor was formed in the absence of air, i.e., even when the solution had been irradiated in a quartz test tube, completely filled and tightly stoppered. If the irradiated material is allowed to stand for some time the odor diminishes and finally disappears.

The odor has been characterized variously as a "peculiar strong odor" (5) or a "burning smell" (6). At first Mond found that this smell was common to all plasma proteins. However, he reported later (7) that when using purified serum albumin and ovalbumin irradiation produced an odor in the latter only.

Color. All of the proteins, whether dry or in solution, became more or less yellow under the influence of the rays, the depth of color depending principally upon the duration of the exposure. Gliadin behaved similarly even though in alcoholic solution. The color formation seemed to parallel the production of an odor, and occurred under exactly the same conditions.

Dreyer and Hanssen (8), Chalupecký (9), Schanz (10), and Mond (6), using blood- or egg-protein solutions, all noted the appearance of a yellowish color upon irradiation. According to Schanz alkalinity favored the deepening of the color; acid solutions, in which a heavy precipitate came down, showed no alteration. Moreover, this author found that prolonged heating at 45°C. for six days produced the same sort of effect as a few hours of exposure to radiations from a mercury arc.

Optical rotation.[5] Edestin in 10 per cent NaCl solution, gliadin in 70 per cent alcohol, and egg white from which the globulin precipitated by dilution had been filtered, all showed definite increases in levorotation when exposed to the rays from the mercury arc. The change in the specific rotation varied directly with the length of exposure. The definiteness of the results obtained in the few experiments tried makes it appear that the optical rotation may be a good indicator of the effect of irradiation provided sufficient attention is given to the control of the variable factors.

Very few observations have been made upon changes in optical properties of proteins due to irradiation. Chalupecký (9) demonstrated qualitatively that the

[5] Thanks are due the Department of Chemistry, Mount Holyoke College; for the use of apparatus necessary for these determinations.

rotation of a solution of egg white was increased by exposure to ultra violet rays. Using apparently the visible light only from a carbon arc Young (11) has reported an increase of 3.78° in the specific rotation of thrice crystallized ovalbumin.

Viscosity and surface tension. No quantitative determinations of either of these properties were made. However, differences in viscosity were observed frequently. It was especially noted that irradiated alcoholic solutions of gliadin always filtered much more readily than a non-radiated solution which, according to quantitative measurements, had the same concentration. The effects may have been determined in some measure by accompanying changes in pH of the solutions; they cannot, however, entirely account for the phenomena. From table 1 it will be seen that film formation was not of universal occurrence in all these protein solutions, but varied in much the same way as the appearance of a coagulum. It would be interesting to determine whether a marked decrease in surface tension occurs simultaneously with film formation.

Dreyer and Hanssen (8) pointed out in 1907 that the formation of these surface films must mean augmented viscosity at the surface. Either ultra violet or Röntgen rays will cause a definite increase in viscosity and a decrease of surface tension of solutions of various blood proteins according to measurements made by Mond (6) and by Wels (12). The amount of change in each case was shown to be influenced by the pH of the solutions. Young (11) found that effects, similar but of much smaller magnitude, were produced in very pure crystallized ovalbumin solutions exposed to a carbon arc from which the longest rays had been filtered out by water and the shortest by glass. On the other hand, Clark (13) has found that purified ovalbumin at pH 6.0 shows no change in viscosity but a marked decrease in surface tension.

The formation of a visible coagulum during irradiation. Although this phenomenon has been the one most frequently mentioned in the literature it has varied more than any other property when a series of different proteins in solution are exposed to the effects of rays from a mercury vapor arc. The only general statement that can be made concerning the action of all the solutions in this respect is that when flocculation occurred its amount increased directly with the duration of the exposure. It should be noted also that in many of the solutions the coagulum continued to deposit at room temperature for several hours after exposure. Unfortunately observations of this particular phenomenon were not recorded for the whole series of proteins. However, this delayed effect must be considered when any quantitative measurements are to be made on the coagulum.

From table 1 two facts may be noted especially: 1, no relation appears to exist between the flocculation and the extent of chemical change which radiation has caused within the protein molecule, if a decrease in the heat-coagulation temperature and changes in viscosity, color and other properties are indicators of such action; 2, there is as much variation within the group of vegetable globulins as within the whole series of proteins.

TABLE 1

Sample protocols showing the effects of radiations from a mercury vapor arc on proteins

PROTEIN	TEMPERATURE	pH CHANGE	ODOR	COLOR CHANGE	FILM	COAGULUM	COAGULATION TEMPERATURE			FLOCCULATION		
							Unradiated	Radiated	Decrease	Unradiated	Radiated	Decrease
	°C.	pH					°C.	°C.	°C.	°C.	°C.	°C.
Ovalbumin	10.0–	−0.1	+++	+	−	−	58.5	47.0	11.5	75	69	6
Hemocyanin	11.8–15.5	−0.7*	++	+++	−	−	64.5,66.5	44.0	20.5,22.5	82,85	70	12,15
Lactalbumin	10.0	−0.2	+	++	−	−	71.0	53.0	18.0	86	64	22
Edestin	12.2–15.5	−0.2	+++	+	+	+++	70.0,71.0	51.0	19.0,20.0	92,93	83,85	9,8
Excelsin	12.0–17.5	+0.5	++++	++	+	++	72.0	53.0	18.5	94	81	13
Phaseolin	17.0–19.0	−0.2	+++	+	−	−	83.0	74.5	8.5	−	−	−
Flaxseed	11.0–15.5	+0.2	+++	+	+	++	70.0	50.5	19.5	95	87	8
Canavalin	11.0–15.5	−0.1	+	+?	−	−	79.0	56.0	23.0	−	−	−
Arachin	12.2–15.5	−0.2	+	+	+	+++	94.0	53.0	41.0	−	88	?
Lima bean	14.5–18.5	−0.1	++	++	−	−	74.0	64.5	9.5	−	−	−
Cantaloupe	14.5–18.5	−0.1	+	+	−	+++	68.0	56.0	12.0	99	99	0
Tomato	10.0–	+0.7	+++	++	+	++	68.5	52.5	16.0	90	73	17

Voltage: 58.
Amperage: 3.5
Distance from arc: 10 cm.
Length of exposure: 15 minutes.
Containers: Open dishes 75 mm. in diameter.
Depth of solution: 3 mm.
Solvent: 10 per cent NaCl.
Concentration of solution: 0.5 per cent or less.
pH of unradiated solution: 6.0.

* The pH before radiation was 6.7 instead of 6.0

When all of the data for each protein are compared it is evident that the presence or absence of a large amount of coagulum in the solution after radiation is a definite characteristic. Under the experimental conditions outlined above, some of the factors which influence this "aggregation" or flocculation during exposure to the arc are: the temperature during and after exposure; the concentration of the protein; the electrolyte content of the solution; and its hydrogen ion concentration. In 41 experiments with crystallized ovalbumin the temperature range extended from less than 10°C. to 21°C., and yet there was never more than a faint turbidity produced in the solution. Nor can the concentration of the protein be the main causative factor for the dissimilar results with different proteins. A length of exposure similar to one which failed to produce more than a very slight clouding in a 0.52 per cent solution of ovalbumin, caused a heavy precipitate to appear in an edestin solution of practically the same concentration (0.55 per cent). If electrolytes are essential to the formation of a coagulum, sodium chloride is not the effective one, because no more precipitate was formed when this salt was present to the extent of 10 per cent than when pure water solutions of electrolyte-free ovalbumin and hemocyanin were used.

The amount of coagulum indicated in table 1 as formed at pH 6.0 is very typical of the behavior of each protein in many other experiments where the pH varied from 5.4 to 7.6. However, this is not a sufficiently wide range of hydrogen ion concentration from which to draw any conclusions. Clark (13) has shown recently that the determining factor in the amount of coagulum formed is not the hydrogen ion concentration *per se* but the iso-electric point. This investigator found that ultra violet radiations caused maximum precipitation to occur in solutions of purified ovalbumin at the iso-electric point, and that on either side of it a gradual decrease in amount took place until no effect was visible. Our results confirm this observation to a certain extent. Ovalbumin which has an iso-electric point of pH 4.8 failed to precipitate at 6.0 or even at 5.4. On the other hand, edestin produced an abundant precipitate anywhere within the range 5.4 to 7.6, which includes the iso-electric point attributed to this protein. It is, however, difficult to explain why the extremely pure hemocyanin failed to form any coagulum at all when exposed at pH 6.7 or 6.8, although its iso-electric point is about 6.5. This fact is especially to be wondered at when it is noted that as a result of the exposure the acidity of the solution increased to about pH 6.0.

Whether these irregularities in the formation of a coagulum as a result of exposure to ultra violet radiations will persist as characteristic properties or whether they will disappear when these proteins are examined with especial reference to their iso-electric points, cannot be predicted at present. It must be emphasized, however, that visible coagulation, or the

lack of it, cannot be considered as indicative of the extent of the effect of radiant energy upon the proteins themselves.

Although, as mentioned above, many investigators have been impressed with the so-called coagulation of proteins by "ultra violet light" a detailed citation of these observations does not seem necessary since the experimental conditions have been so variously defined. Clark, Mond, Bovie and Young have all pointed out that the photochemical change in the protein itself occurs as a process quite distinct from any visible alteration in the dispersion of the protein. In this connection it may be of interest to recall the work of Nordenson (14), one of several who have examined the effects of radiations on colloidal systems less complex than proteins. By means of ultramicroscopic observations he found that exposure to a quartz mercury arc of 220 volts and 3.5 amperes caused a slow coagulation of colloidal gold. The gold was found to have undergone no chemical alteration as a result of the radiation and the conclusion was drawn that the change was purely physical. Furthermore, the coagulation took place whether the charge on the gold particles was positive or negative.

Effect of radiations upon the coagulation temperature of proteins. Chick and Martin, and others, have pointed out that the coagulation temperature is not a fixed property of proteins but depends upon a variety of factors. However, they state (15): "the temperature at which under precisely similar conditons a precipitate is first visible may be useful in differentiating proteins." This method has proved advantageous also as an indicator of the effect of radiant energy upon proteins. Every effort was made to maintain the experimental conditions as constant as possible. Two tubes of the protein solution in question, differing only in that the contents of one had been exposed to the arc, were heated side by side in a well-stirred double oil-bath. The rate of heating was as uniform as possible and always slow. The first appearance of turbidity was called the "coagulation temperature."

If heated subsequent to exposure to the arc, every protein investigated, provided it was initially heat-coagulable, both became cloudy and flocculated at a lower temperature than the non-radiated material. It will be observed in table 1 that this decrease in coagulation temperature was marked in each case. In fact flocculation of the irradiated solutions sometimes occurred before the control had even become turbid. Since the lowering of the coagulation temperature was relatively greater than the decrease in the temperature at which flocculation occurred the "coagulation zone" was broadened, as has been observed by Mond (6) to be the case with blood proteins. In general the amount of decrease shown in table 1 was roughly characteristic of each protein. One could predict, for instance, that under comparable conditions phaseolin would have its coagulation temperature decidedly less affected by irradiation than edestin. The experimental work taken as a whole covered the pH range 5.4 to 7.7. The temperatures at which the exposures were made varied from 9.5°C. to 23°C.

While the greater number of observations (6), (9), (16), (17) already made indicate that a decrease in coagulation temperature may be expected as a result of protein irradiation, still under certain conditions thus far not clearly understood the reverse takes place. Several years ago Dreyer and Hanssen (8) found that radiated fibrinogen was coagulated by heat at a higher temperature than the non-irradiated material. On the other hand, Mond reported a lower coagulation temperature for this protein, and Howell (18) failure to coagulate at all. According to Mond the extent of decrease in the coagulation temperature of serum albumin is related to its iso-electric point (4.7), being much greater on the alkaline side and having its maximum at pH 5.4. It is unfortunate that the experiment was not continued beyond pH 6.0. A phenomenon called "desensitization" by Clark (13), which seems to be related to the purity of the protein, has been reported also by Bovie and Woolpert (19). These latter investigators observed (and this has been confirmed incidentally by us) that alkaline solutions of diluted egg-white previously irradiated would not coagulate even when heated to 100°. Furthermore this coagulation-preventing factor was quite powerful, for a small amount of irradiated solution added to a large volume of non-irradiated prevented the coagulation of the latter. Clark was able to establish definitely that especially purified ovalbumin could not be desensitized to heat by irradiation at room temperature, but that when in combination with a protein-like impurity which could be so desensitized, was itself affected similarly by the rays. However, under certain conditions even the very pure ovalbumin could be desensitized. It is certain that some factor outside our experimental conditions must cause this remarkable effect, for it was not observed in any of the work with our series of isolated proteins.

The change in pH resulting from irradiation. It seems unnecessary to discuss the various, apparently conflicting observations which have been reported concerning acidity changes brought about in solutions by ultra violet radiations. Clark (20) (13) found an increase in the pH of ovalbumin solutions, whether the initial hydrogen ion concentration was such as to give a pH of 3.6, 6.0, or 7.2. On the other hand Mond (7) and Young (11) concluded from their data that a correlation exists between the isoelectric point and the direction of the change. Acid solutions of proteins tend to become less so under the influence of the rays, while those on the alkaline side of the isoelectric point to begin with become more acid. The region of least change is just acid to the isoelectric point according to Mond, while Young found it to be slightly on the alkaline side.

Since our pH observations were recorded merely as an incidental part of the data defining experimental conditions, colorimetric determinations only were made. Whenever the solutions were colored or contained an appreciable amount of salts a comparator was used. As usual the irradiated and non-irradiated materials were compared side by side. Emphasis was placed upon the occurrence of a *change* in reaction rather than upon the determination of the absolute hydrogen ion concentration.

When our protein solutions were exposed to rays from the 165 volt mercury arc at a distance of 27 cm., constant variations in pH were not obtained. See table 2. These changes which followed the short exposures of 5 or 15 minutes were so small, amounting usually to about 0.2 pH, that they could not have had any important bearing upon the marked modifications which occurred in the properties of the proteins.

However, when it became necessary to use the smaller arc, the distance was shortened to 10 cm. and the time extended to 30 minutes, in order to get comparable effects. To our surprise the protein solutions now always became decidedly acid after exposure, although they showed less marked changes in properties than with the other arc. This increased acidity could be much lessened by shortening the duration of the exposure to 15 minutes. It was further observed that dry proteins, irradiated for

TABLE 2

pH variations resulting from exposure of protein solutions to a quartz mercury vapor arc

PROTEIN	NUMBER OF EXPERIMENTS	NO CHANGE IN pH	INCREASED pH	DECREASED pH
Ovalbumin	28	19	3	6
Edestin	28	9	15	4
Vegetable globulins other than edestin	16	8	2	6
Miscellaneous non-purified proteins	16	9	2	5
Summary	88	45	22	21

Voltage: 165.
Distance: 27 cm.

TABLE 3

Decrease in the pH of water caused by radiations from a quartz mercury arc

KIND OF WATER	pH OF NON-RADIATED WATER	pH OF RADIATED WATER
Distilled water A (unboiled)	6.4	6.1
Distilled water A (unboiled)	6.4	5.9
Distilled water B (unboiled)	6.2	5.9
Distilled water C (triply distilled)	6.8	6.1
Distilled water A (boiled)	7.0	5.8
Distilled water B (boiled)	7.0	5.9
Distilled water B (boiled)	7.0+	6.2
Distilled boiled B (boiled plus CO_2 drawn through)	6.0	5.6

Voltage: 58.
Amperage: 3.5.
Distance from arc: About 6 cm.
Time of exposure: 15 minutes.
Indicator: Brom cresol purple.

as much as two hours, never formed a more acid solution than the non-irradiated material treated similarly; but indeed tended to become more alkaline. Clearly this hydrogen ion increase was due to some change in the solvent rather than in the protein molecule itself. It was then found that water, even when triply distilled through glass and rayed immediately, showed exactly the same increase in acidity as did the protein solutions. The extent of this pH change in water may be observed from table 3. Three indicators were used to confirm the fact that a change had occurred.

This decrease in the pH of distilled water could scarcely be due to the absorption of the oxides of nitrogen or other acid forming gases from the air for no appreciable difference in pH could be detected when the air from directly around the lamp was drawn through a protein solution for 30 minutes. Moreover a quartz test tube, filled to the brim and stoppered tightly so that no air bubbles were visible, allowed just as much acid formation, as did the open dish. Since the water contained in a similar quartz tube which had been enclosed in a larger pyrex glass tube failed to show any alteration whatsoever in pH after irradiation, it seems probable that the effect was due to those wave lengths filtered out by pyrex glass.

Tian (21) and Kernbaum (22) have shown that wavelengths of less than 1900 Å can slowly decompose water according to the equation

$$2H_2O \rightarrow H_2O_2 + H_2$$

Luckeish (23) points out that the mercury vapor arc emits several lines in the region of 1850 Å. Indeed this is given as a text book method for the preparation of hydrogen peroxide. Fernau (24) and others have demonstrated that not only is H_2O_2 formed in aqueous solutions under the influence of Röntgen, Becquerel, or ultra violet rays, but that it alone can produce the same kind of effects on the properties of sugar and protein solutions as these radiations. Fernau was able to show, however, that prevention of the formation of hydrogen peroxide within the solutions during irradiation did not lessen appreciably the amount of the change occurring therein.

The rôle of water, electrolytes, and air in the effects caused by the radiations. The presence of any quantity of water is not a condition necessary to the production of these changes in the proteins due to radiant energy. Ovalbumin and edestin after irradiation in the powdered form gave off the characteristic strong odor, acquired a distinctly yellowish tinge, and if dissolved subsequent to the exposure, coagulated at a lower temperature than similar, though non-irradiated material. This was true even when proteins desiccated by the method of Benedict and Manning (25) were irradiated. A longer exposure was required to produce an effect comparable to that occurring in solutions. Material dried similarly, but irradiated in glass showed none of the characteristic changes. Bovie has noted briefly (26) that "albumen and other proteins, dried in vacuo, are readily decomposed by ultra-violet rays." This, then, must represent one respect in which proteins react differently to heat radiations than to those of short wave length.

Electrolyte-free solutions of proteins respond to the action of the rays as readily as those containing sodium chloride or ammonium sulphate. No difference could be demonstrated between the results obtained when hemocyanin, which had been dialysed in a vacuum until free from chlo-

rides and sulphates, and ovalbumin, giving no test for sulphates, were radiated in pure distilled water or in 10 per cent NaCl.

That contact with a large amount of air was not concerned with the effect of the radiations was shown in two ways: *a*, The air from directly around the arc drawn through the protein solutions as mentioned above failed to cause any change to take place within them. *b*, A quartz test tube was completely filled with edestin solution and stoppered so that air bubbles were excluded and then irradiated as usual. The solution acted in the same way as if it had been exposed in an open dish, or through quartz but with a large air space over the material. Bovie has shown that evacuation of the solution causes no alteration in the effects of the rays.

It seems safe, therefore, to conclude that neither water nor air are factors essential to the described action of ultra violet radiations upon proteins. The possible influence of electrolytes and of changes in pH has not yet been studied in sufficient detail to warrant a final statement.

SUMMARY

A series of twelve purified proteins have been exposed to rays from a quartz mercury vapor arc with the following results:

The same characteristic odor was produced in all of the proteins. All of them tended to assume a yellowish tint as a result of the irradiation. The temperature at which the proteins coagulated when heated was lowered in every case, without exception. The above changes appeared to occur independent of the presence of water or air in the environment of the protein. As yet no evidence has been obtained to indicate that electrolytes play a determining rôle. Edestin, gliadin, and diluted egg albumen appeared to have their specific rotation increased by the action of the rays. The appearance of a coagulum within solutions of these proteins during irradiation was by no means a universal occurrence, and was characteristic of each protein under our experimental conditions. A surface film was not formed on all of these protein solutions during exposure to the arc. Under certain experimental conditions the pH, not only of the protein solutions themselves but of distilled water, can be decreased decidedly as a result of absorption of radiations from the arc.

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THE EFFECT OF VARIOUS SUGARS (AND OF ADRENALIN AND PITUITRIN) IN RESTORING THE SHIVERING REFLEX

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When the blood sugar concentration of a cat or a dog is lowered by means of insulin to about 45 mgm. per 100 cc., the animal loses its capacity for shivering. The subsequent administration of glucose causes this reflex to reappear (the authors, 1925). The experiments recorded here were undertaken with a view to determine whether the injection of other sugars into hypoglycemic animals would produce similar effects.

The general mode of procedure was as follows: Anesthesia was induced in a cat or dog by means of amytal. The animal was then tested for shivering by exposure to cold. It was found that some of the cats did not respond to moderate stimulation; such animals were discarded. For the determination of the normal sugar concentration a sample of blood was obtained either from the saphenous or from the external jugular vein, the Folin-Wu blood sugar method being used. Insulin was then injected subcutaneously, the dose being 5 units per kilogram of body weight. Three or 4 hours later shivering could not be elicited even by wetting the animal and placing it in a cold draught. A blood sample was taken at or soon after this time. An intravenous injection of one of the sugars whose effect was to be determined was then given. The concentration of the sugar solution was 20 per cent in the case of the monosaccharides and 40 per cent in the case of the disaccharides. The dose varied from 1 gram to 12 grams per kilogram of body weight. The animal was observed closely and tested frequently for the reappearance of shivering. If no effect was noted in from 30 to 60 minutes, glucose was given intravenously to control the experiment.

In these experiments the onset of shivering was always preceded by a marked change in the condition of the animal. The hypoglycemic dog or cat under light amytal anesthesia exhibits no body movements apart from those of respiration; the heart rate is diminished, and the blood pressure is low. The first apparent change after the injection of the sugar is usually a deep sighing respiration. This is followed by twitching of the muscles

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of the head and neck, and by feeble progression movements, which gradually increase in amplitude. Simultaneously the respirations become deeper and more rapid, the heart rate increases, and the blood pressure rises. Shivering usually commences in less than 1 minute after the first of these changes is noted. Although shivering is at first barely perceptible, and involves only the flank, it rapidly increases in violence and becomes general.

Monosaccharides. The following monosaccharides were used: fructose, mannose, galactose, and dioxyacetone[2] (glycerulose). As shown in table 1, the administration of any one of these sugars to a hypoglycemic animal causes the reappearance of the shivering reflex. The time elapsing between the injection of the carbohydrate and the onset of shivering varied from 3 minutes in the case of dioxyacetone and of mannose, to 23 minutes in the case of galactose. The corresponding time for glucose is 1 to 3 minutes.

Maltose. Two cats and 2 dogs received maltose after hypoglycemia had been induced. As shown in table 1, shivering reappeared in from 4 to 33 minutes after the injection. This effect was probably due to the formation of glucose by hydrolysis of the maltose in the blood stream, for, as Tebb (1894) and others have shown, a maltase is present in blood serum. This view was confirmed by determining the glucose and maltose content of blood samples taken at frequent intervals after the intravenous injection of 2 grams of maltose per kilogram into a hypoglycemic dog.

The details of the experiment were as follows:

Dog 36, weight 6.6 kgm.

10:44. Animal received 0.4 cc. amytal per kgm.
11:20. Animal was shivering; normal blood sugar 122 mgm. per cent.
11:25. Animal received 5 units insulin per kgm.
4:05. Shivering was abolished; blood sugar 28 mgm.
4:25. Animal received 2 gm. maltose per kgm. intravenously.
4:50. Animal showed progression movements; blood glucose 98 mgm.; blood maltose 215 mgm.
4:58. Shivering began.
5:00. Blood glucose 84 mgm.; blood maltose 146 mgm.
5:10. Shivering was vigorous. Blood glucose 105 mgm.; blood maltose 29 mgm.
5:20. Blood no longer contained maltose; blood glucose 104 mgm.
5:40. Blood glucose 82 mgm.
6:45. Shivering could not be induced; blood glucose 42 mgm.

The glucose concentration in the blood of this animal was 84 mgm. per cent at the time when shivering reappeared, whereas it was only 28 mgm. per cent when the maltose was injected. During the 33 minutes elapsing between the injection and the development of shivering, the maltose content of the blood must have been greater than 146 mgm. per 100 cc. If maltose itself could have caused the reappearance of shivering, it should

[2] Kindly supplied by the Mallinckrodt Chemical Works.

TABLE 1

	NORMAL BLOOD SUGAR	BLOOD SUGAR AFTER SHIVERING STOPPED	SUGAR INJECTED	DOSE	EFFECT	SUGAR INJECTED SUBSEQUENTLY AS CONTROL	DOSE	EFFECT
	mgm. per cent	*mgm. per cent*		*grams per kgm.*			*grams per kgm.*	
Cat 18	125	44	Fructose	6.0	Shivering began in $9\frac{1}{2}$ minutes			
Cat 35	200	39	Mannose	2.0	Shivering in 4 minutes			
Dog 32		41	Mannose	2.0	Shivering in 3 minutes			
Cat 36	119	41	Galactose	2.0	Shivering in $22\frac{1}{2}$ minutes			
Dog 31		36	Galactose	2.0	Shivering in 20 minutes			
Cat 17	129	38	Dioxyacetone	3.0	Shivering in 3 minutes			
Cat 15	94	31	Maltose	11.4	Shivering in 12 minutes			
Dog 30	120	33	Maltose	2.0	Shivering in 11 minutes			
Cat 47	101	31	Maltose	1.0	Shivering in 4 minutes			
Dog 36	122	28	Maltose	2.0	Shivering in 33 minutes			
Cat 24	108	37	Sucrose	11.4	Shivering in 15 minutes			
Cat 21	113	Lost	Sucrose	11.4	No effect after 31 minutes	Glucose	6	No effect in 24 minutes
Cat 25	136	39	Sucrose	11.4	No effect after 36 minutes	Glucose	1	No effect in 55 minutes
Cat 31	134	36	Sucrose	11.4	No effect in 30 minutes			
Cat 13	117	50	Sucrose	3.8	No effect after 31 minutes	Glucose	2	Shivering in 6 minutes
Cat 28	150	39	Sucrose	1.9	No effect in 52 minutes	Glucose	1	Shivering in 4 minutes
Cat 38		23	Sucrose	4.0	Shivering in 22 minutes			
Cat 41		23	Sucrose	2.0	Shivering in 14 minutes			
Cat 42		23	Sucrose	2.0	Shivering in 9 minutes			
Cat 45	143	30	Sucrose	2.0	No effect in 1 hour			
Cat 16	92	32	Lactose	12.0	No effect after 40 minutes	Glucose (subcutaneously)	6	Shivering in 3 minutes
Cat 19	125	35	Lactose	12.0	No effect after 31 minutes	Glucose	6	Shivering in 44 minutes
Cat 26	99	46	Lactose	12.0	Shivering in 30 minutes			
Cat 34	204	22	Lactose	2.0	No effect in 14 minutes	Glucose	2	Shivering in 5 minutes
Cat 50		20 or less	Lactose	2.0	No effect in 64 minutes	Glucose	2	Shivering in 8 minutes
Cat 51		20 or less	Lactose	2.0	No effect in 151 minutes	Dioxyacetone	2	Shivering in 2 minutes

have done so during these 33 minutes. It is therefore reasonable to conclude that the development of shivering was due to glucose, and not to maltose. Fifty-five minutes after the maltose injection the blood no longer contained any maltose. The disappearance of this sugar was due in part to its conversion to glucose, and in part to its excretion by the kidneys. The glucose concentration in the blood at the time when the maltose had disappeared was 104 mgm. per cent, and the animal was shivering violently. One hour and 25 minutes later the dog failed to shiver on exposure to cold. It was found that the blood glucose concentration had fallen to 42 mgm. per cent.

The method evolved for the determination of maltose in the presence of glucose is based upon the difference between the reducing powers of these two sugars. Preliminary experiments showed that the reducing power of maltose to the Folin-Wu cupric reagent was 60.7 per cent of that of glucose. The reducing power of the tungstic acid blood filtrate in which both maltose and glucose were to be determined, was estimated by the Folin-Wu blood sugar method before and after acid hydrolysis. From these two values the concentrations of maltose and of glucose present in the blood could readily be calculated. The hydrolysis was carried out as follows. One cubic centimeter of the blood filtrate was pipetted into a Folin-Wu blood sugar tube, and 0.5 cc. of normal hydrochloric acid added. The tube was placed in a boiling water-bath for 50 minutes. After cooling, the acid was neutralized with 0.5 cc. of normal sodium hydroxide solution. Folin-Wu cupric reagent was then added, and the determination of the reducing power completed in the usual way. Control experiments with maltose solutions showed that the method just described is effective in producing complete hydrolysis of the maltose. The blood samples were analyzed as rapidly as possible after removal from the animal, in order to minimize error due to conversion of maltose to glucose.

Folin and Berglund (1922) pointed out that acid hydrolysis may decrease the reducing power of filtrate from whole blood, although an increase is more often obtained. In the experiment quoted it was found that the reducing powers of the filtrates obtained from the blood samples taken at 5:20 and 5:40 p.m. were decreased to the extent of 10 mgm. per cent by hydrolysis. Had a similar decrease occurred when the filtrates containing maltose were hydrolysed, the values for maltose would be too low, and those for glucose too high. Such a change in these figures would not weaken in any way the evidence leading to the conclusion that the reappearance of shivering following maltose administration is due not to maltose as such, but to the glucose derived from it.

Sucrose. The sucrose used in these experiments was the purest obtainable, containing less than 0.1 per cent of invert sugar. In the earlier experiments 11.4 grams of sucrose per kilogram of body weight were given

intravenously to 3 animals after shivering had ceased. As shown in table 1, cat 24 began to shiver 15 minutes after the injection. Shivering did not develop in cats 21 and 25 even after 31 and 36 minutes respectively. These two animals then received glucose intravenously. Shivering did not reappear, nor did the condition of the animals change in any way. Since about 150 cc. of hypertonic solution had been injected, it was considered that the failure of the animals to respond to glucose was due to the great increase in volume of the circulating blood. Accordingly, 7 hypoglycemic cats received intravenously amounts of sucrose varying from 1.9 grams to 4 grams per kilogram of body weight. In 4 of these animals sucrose had no effect, whereas shivering reappeared in the other 3 in from

TABLE 2

	NORMAL BLOOD SUGAR	BLOOD SUGAR AFTER SHIVERING STOPPED	AMOUNT OF SUCROSE INJECTED	BLOOD SUGAR WHEN SHIVERING BEGAN	TIME BETWEEN INJECTION AND SHIVERING	REMARKS
	mgm. per cent	*mgm. per cent*	*grams per kgm.*	*mgm. per cent*	*minutes*	
Cat 24	108	37	11.4		15	6 minutes after shivering began, blood sugar 107 mgm.
Cat 25	136	39	11.4			No shivering. Blood sugar 31 minutes after injection, 50 mgm.
Cat 38		23	4	42	22	
Cat 42		23	2	30	9	Shivering violent 5 minutes after its onset. Blood sugar at this time, 40 mgm.
Cat 45	143	30	2			No shivering. 15 minutes after injection, blood sugar 35 mgm. 45 minutes later blood sugar, 20 mgm. or less

9 to 22 minutes after the injection (table 1). Subsequent injection of glucose into the animals which did not respond to sucrose promptly caused them to shiver. In order to be certain that sucrose was present in the blood of the cats in which this sugar produced no shivering reaction, the sucrose content of blood samples taken from one of these animals (cat 45) was determined by the Folin-Wu method after hydrolysis with hydrochloric acid. The blood of this cat contained 446 mgm. per cent of sucrose 15 minutes after, and 96 mgm. per cent 1 hour after the injection of 2 grams per kilogram.

It seemed probable that the occurrence of shivering after sucrose injection was due to the formation of invert sugar, since according to Abderhalden (1914) an invertase may appear in the blood stream a few minutes

after the parenteral administration of sucrose. This was confirmed by experiments in which samples of blood were taken at frequent intervals after sucrose injection, and their content of glucose plus invert sugar was determined. As shown in table 2, the concentration of reducing sugar in the blood was found to be slightly increased after the animals received sucrose. Moreover, in the cats which shivered the increase was more marked than in those which did not. This finding would indicate that shivering follows sucrose injection only when a sufficient amount of the sugar has been inverted. Further evidence that sucrose as such does not cause the restoration of the shivering reflex is furnished by the following experiment. A hypoglycemic cat received repeated injections of small amounts of sucrose. In this way a considerable concentration of blood sucrose was maintained for a period of 2 hours and 57 minutes. Shivering did not occur during this time and the reducing sugar concentration did not rise appreciably. The injection of glucose produced shivering at the end of 3 minutes.

Lactose. Six cats received lactose intravenously. In only one case was shivering observed. The other animals did not shiver during observation periods up to 151 minutes following lactose administration. Four of these animals were subsequently given glucose and one was given dioxyacetone; shivering occurred in every case. It is perhaps worthy of note that the cat which shivered after the injection of lactose was a young animal. It has been reported (Weinland, 1907) that a lactase is occasionally present in the blood of young animals.

Adrenalin. Six hypoglycemic cats received 2 cc. to 6 cc. of adrenalin (1:1000 solution) subcutaneously. To facilitate absorption the drug was in each case injected in several places. Shivering returned in every case, the blood sugar level at which this occurred varying between 51 mgm. and 114 mgm. per cent. The time elapsing between the injection of adrenalin and the onset of shivering varied greatly in different cats—from 4 to 65 minutes.

Pituitrin. The effect of pituitrin in restoring the shivering reflex was investigated in 1 dog and 1 cat. Although the blood sugar rose only 3 mgm. and 11 mgm. per cent respectively in these animals, shivering developed in both cases 30 minutes after injection of the drug. The blood sugar level when shivering returned in the dog was 33 mgm., and in the cat 31 mgm. per cent. These facts may indicate that the restoration of shivering as a result of injecting pituitrin is due to some mechanism other than the increase in the glucose concentration of the blood. Burn (1923) has pointed out that pituitrin is antagonistic to insulin.

Abolition of convulsions. It has been reported by Noble and Macleod (1923) that glucose is the only sugar which can definitely counteract the symptoms that accompany insulin hypoglycemia in the rabbit: adminis-

tration of fructose, of galactose, or of maltose may be followed by temporary slight improvement in the symptoms; mannose is almost as efficient as glucose, while sucrose and lactose have no apparent effect. Since these observations concerning the efficacy of the different sugars in abolishing insulin convulsions do not correspond with some of our findings with respect to the restoration of the shivering reflex, it was decided to reinvestigate the effect of these carbohydrates upon insulin convulsions. The experiments were carried out on cats, in which animals the injection of insulin always produces well-marked convulsions. The sugars were injected subcutaneously in amounts varying from 3 to 4 grams per kilogram, except in the case of sucrose, where doses as large as 10 grams per kilogram were administered. It was found that dioxyacetone, mannose, fructose, galactose, and maltose produced complete recovery. The cat which received maltose developed convulsions 7 or 8 hours after the injection; nevertheless it recovered. All the animals were apparently normal on the following morning. Sucrose and lactose were ineffective in abolishing convulsions. The discrepancies between these findings and those of Noble and Macleod may be due to the fact that different experimental animals were used. Further, their animals received massive doses of insulin, yet the amounts of sugar subsequently injected were not correspondingly large.

It will be noted that those sugars which cause shivering to reappear also abolish insulin convulsions. Sucrose and lactose seem to be exceptions, but, as has been pointed out, these sugars as such are incapable of causing shivering, and would, therefore, not be expected to abolish insulin convulsions unless hydrolysed in the blood stream. In the experiments upon the abolition of insulin convulsions, it is possible that the sucrose injected was hydrolysed but not to a sufficient extent to produce recovery; for the rise of reducing sugar concentration in the blood following sucrose administration is small.

CONCLUSIONS

1. Dioxyacetone, fructose, mannose, galactose, as well as glucose, restore the shivering reflex after it has been abolished by insulin hypoglycemia.

2. Shivering is induced in hypoglycemic cats after the injection of maltose, and sometimes after the injection of sucrose and of lactose These sugars as such are inactive, and the induction of shivering is due to the products of their hydrolysis.

3. Administration of adrenalin or of pituitrin to hypoglycemic cats causes shivering to reappear.

4. Dioxyacetone, fructose, mannose, galactose, and maltose, as well as glucose, abolish insulin convulsions in cats. Sucrose and lactose are ineffective.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

XIII. The Liver as a Site of Bilirubin Formation

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The results of experiments previously reported showed conclusively that bilirubin was added to the blood as it traversed the spleen and bone marrow (1), (2) and that therefore it was formed in these two tissues. Bilirubin was not added to the blood while the latter was passing through any of the other tissues studied, which included practically all the tissues of the body except the liver and certain small structures. We wish now to present data proving that bilirubin is also formed in the liver.

Method of investigation. The method employed in the previous study consisted in obtaining specimens of venous and arterial blood of the organs under investigation and determining the relative concentration of bilirubin present by means of the spectrophotometer. Several difficulties presented themselves in the application of this method to the liver. The liver not only has a double source of blood supply, to one of which the spleen adds bilirubin, but as the sole site for the normal excretion of bilirubin, it is constantly, although not at a uniform rate, taking bilirubin from the blood stream. Furthermore, the liver drains through several separate vessels into the vena cava where it is quickly mixed with the blood coming from the lower extremities.

These various difficulties were overcome by a procedure which essentially consisted in 1, making the concentration of the bilirubin in the two sources of blood supply to the liver the same by removing the spleen; 2, preventing the liver from excreting bilirubin by removing the gall bladder and ligating the common bile duct, and 3, concluding the vena cava temporarily just caudally to the entrance of the hepatic veins, thus obtaining the blood leaving the liver free from dilution with other blood. As we have previously shown that the blood in the mesenteric artery and vein of the fasting dog contains the same concentration of bilirubin, in order to make the bilirubin content of the hepatic artery and portal vein the same it was only necessary to remove the only organ which added bilirubin to the portal

system, that is, the spleen. Thus the difficulty of the double blood supply to the liver was eliminated. In order to eliminate the variable factor introduced by the excretion of bilirubin by the liver, the gall bladder was removed and the common bile duct ligated. It can be readily seen that if, in the development of the bilirubinemia that follows obstruction of the common bile duct, the blood leaving the liver contains more bilirubin than the blood entering the organ, the excess of pigment must have been formed by the liver. In other words, if, at the time the bilirubin concentration of the arterial blood is increasing, the bilirubin content of the blood in the hepatic veins is greater than that of the portal vein and hepatic artery, some pigment must have been added to the blood stream by the liver. However, in order that the data thus obtained be representative of the processes actually occurring, it is essential that the bilirubin values be noted at various periods after obstruction to the outflow of bile, especially, soon after the obstruction before the duct and capillary systems have become disorganized, and that care be exercised not to squeeze pigment from the liver while the specimen of blood is being obtained. A cava pocket can be readily made of that portion of the cava into which the hepatic veins empty by placing a loose ligature around the vena cava between the entrance of the lumbo-adrenal vein and the inferior hepatic vein, and tightening it just before and during the time the specimen of venous blood is being taken.

The experiments were performed on dogs under ether anesthesia. As we have shown that the bilirubin content of the arterial blood is the same throughout the entire system, specimens of arterial blood were taken from the femoral rather than the hepatic artery as we did not wish to interfere in any way with the blood supply to the liver. A specimen of blood from the femoral artery was obtained to determine the normal content of bilirubin in the arterial blood. Then the abdomen was opened, the gall bladder removed and the common bile duct ligated. After waiting for a variable period for the development of the bilirubinemia following obstruction of the biliary tract, the spleen was removed and the loose ligature passed around the vena cava. Then simultaneously specimens of blood were obtained from the femoral artery and the cava pocket (figs. 1 and 2).

It should also be noted that in some of the experiments the procedure was varied in order to obtain control data. Occasionally a specimen of blood from the portal vein was taken. Specimens were also procured from the femoral vein, and from the splenic vein before removal of the organ. In a few experiments the spleen was not removed but blood was taken from the portal vein at the same time as from the femoral artery and cava pocket. The preparation of the plasma for estimation of the bilirubin as well as the method of estimation with the spectrophotometer was the same as in the previous investigation.

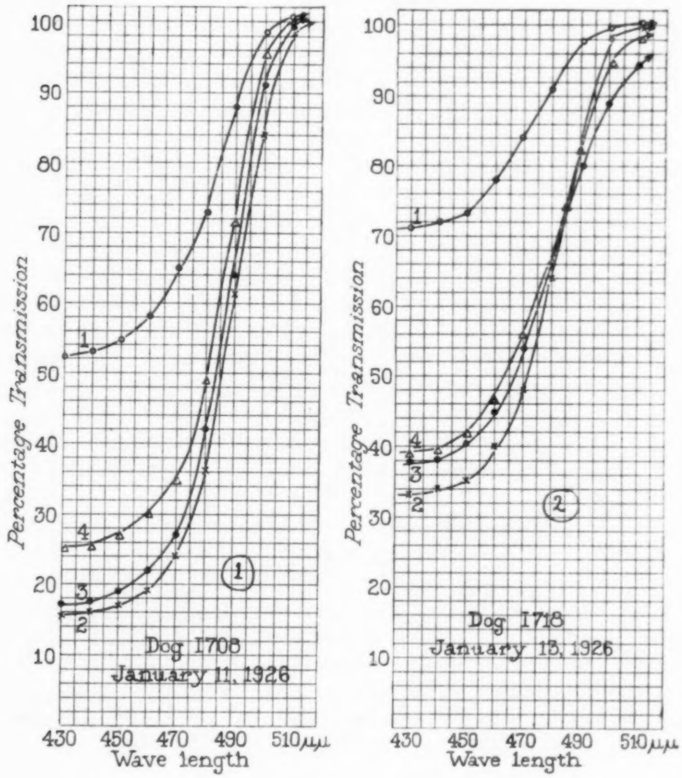

Fig. 1. Curves showing the greater bilirubin content of the blood of the hepatic and splenic veins as compared with the blood of the femoral artery 36 minutes after ligation of the common bile duct and extirpation of the gall bladder. Curve *1*, light transmission curve of the alcoholic extract of plasma from the femoral artery; curve *2*, from the splenic vein 36 minutes after operation; curve *3*, from the hepatic vein 36 minutes after operation; curve *4*, from the femoral artery, 36 minutes after operation.

Fig. 2. Curves showing the greater bilirubin content of the blood of the hepatic vein as compared with the bloods of the femoral artery and portal vein 33 minutes after ligation of the common bile duct and extirpation of the gall bladder. Curve *1*, light transmission curve of the alcoholic extract of plasma from the femoral artery; curve *2*, from the hepatic vein 33 minutes after operation; curve *3*, from the femoral artery 33 minutes after operation; curve *4*, from the portal vein 33 minutes after operation.

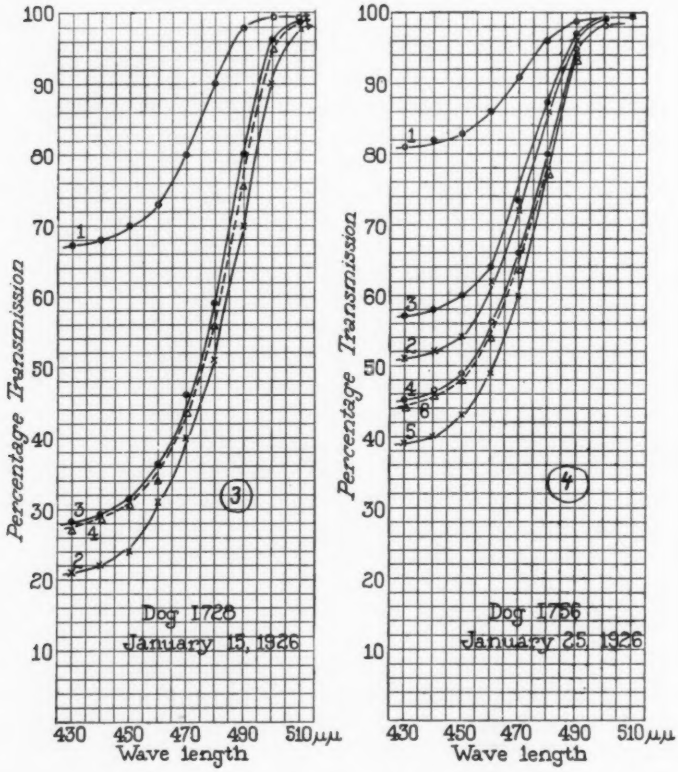

Fig. 3. Curves showing the greater bilirubin content of the blood of the hepatic vein as compared with the bloods of the femoral artery and portal vein 63 minutes after ligation of the common bile duct and extirpation of the gall bladder. Curve *1*, light transmission curve of the alcoholic extract of plasma from the femoral artery; curve *2*, from the hepatic vein 63 minutes after operation; curve *3*, from the femoral artery 63 minutes after operation; curve *4*, from the portal vein 63 minutes after operation.

Fig. 4. Curves showing the greater bilirubin content of the blood of the hepatic vein as compared with the blood of the femoral artery 30 and 60 minutes, respectively, after ligation of the common bile duct and extirpation of the gall bladder. Curve *1*, light transmission curve of the alcoholic extract of the plasma from the femoral artery; curve *2*, from the hepatic vein 30 minutes after operation; curve *3*, from the femoral artery 30 minutes after operation; curve *4*, from the femoral artery 60 minutes after operation; curve *5*, from the hepatic vein 60 minutes after operation; curve *6*, from the portal vein 60 minutes after operation.

Results. The rate of increase of the bilirubin in the blood after removal of the gall bladder and obstruction of the common bile duct was unexpectedly rapid. In some animals there was a measurable increase within fifteen minutes after obstruction of the duct. In most animals it had almost doubled in amount within thirty minutes. When the spleen had been removed or its blood vessels clamped the bilirubin content of the blood of the portal vein was always the same as the bilirubin content of the arterial blood. After ligation of the common bile duct and removal of the gall bladder, as the bilirubin content of the arterial blood was increasing, the bilirubin content of the blood leaving the liver was greater than that of the blood entering the liver. Adequate control observations with regard to securing the specimen of blood at different intervals after obstruction of the biliary tract, with regard to the rate of development of the bilirubinemia, and with regard to the bilirubin content of the blood leaving other organs, all emphasized the major finding, that after conversion of the double blood supply of the liver into a single one as regards bilirubin content and after prevention of excretion of bile pigment through the biliary tract, the bilirubin content of the blood leaving the liver was definitely and measurably greater than that of the blood entering the organ (figs. 3 and 4).

Discussion. The method of transforming the complex condition of the liver with reference to the bilirubin content of the blood entering this organ in relation to that leaving it, into the same simple condition as appertains to other organs, was successful. The prevention of blood from the spleen from entering the portal circulation reduced the dual blood supply of the liver to a unified one as regards bilirubin content of the entering blood. Removal of the gall bladder and obstruction of the common bile duct effectively prevented the loss of bilirubin through the biliary tract. Following these procedures the liver was no different with reference to the bilirubin content of the blood than the other organs we have studied. Under such conditions the bilirubin content of the blood secured from the hepatic veins was consistently greater than that of arterial blood. The liver had added bilirubin to the blood as it traversed the organ.

One of the most surprising results of these experiments was the rapidity with which the bilirubin content of the blood increased after removal of the gall bladder and obstruction of the common bile duct. As a matter of fact this finding was so unexpected that our first experiment was more or less of a failure because we postponed obtaining our blood specimens too long after obstruction. The amount of bilirubin in the blood was then so large that considerable difficulty was experienced, because the amount of bilirubin added to the blood as it passed through the liver was only a very small percentage of the bilirubin in the blood. This same amount of bilirubin added to blood of normal bilirubin content gives a large and unmis-

takable percentage increase. We found also that the rate of accumulation of bilirubin following biliary obstruction is so rapid that if blood specimens from different sources are to be compared with reference to their bilirubin content, they must be secured simultaneously. In all the experiments there was a definite and measurable increase in the bilirubin content of the arterial blood within thirty minutes after obstruction of the biliary outflow. While the actual amount of bilirubin which accumulates in that period is small, it is large compared with the amount present before obstruction. Evidently the secretion of bile pigment by the liver is very easily interfered with in cases of obstruction.

Two possible sources of error in the method are to be considered. It is conceivable that the pigment brought to the liver might accumulate in the liver cells for a short time following obstruction to the biliary outflow and be returned to the blood stream in irregular amounts. If this occurred it might be possible to find an increased amount of bilirubin in the blood of the hepatic veins owing to pigment which had only been stored in the liver. In this event there should be some periods after obstruction in which the bilirubin content of the arterial blood should be increasing and the bilirubin content of the hepatic venous blood be either the same as the arterial blood or actually less. We obtained a specimen of blood at various times after obstruction but not before the bilirubin was increasing in the arterial blood, and in no instance was an actual decrease obtained in the amount of pigment in the blood of the hepatic vein in relation to the arterial blood. It is not necessary for us to consider the debatable question of whether, following obstruction, the bile pigment enters the lymph or blood, except to point out that, because our results are positive with regard to an increase of the bilirubin in the hepatic vein over that in the arterial blood, whatever amount of bile pigment was eliminated through the lymphatics would have enhanced the general results.

The difference between the bilirubin content of the blood and the hepatic vein and in that of the arteries was so definite that some of the bilirubin had unquestionably been added to the blood as it passed through the liver. As we have obtained adequate control data and eliminated the possible sources of error, there would appear to be no doubt that some bilirubin is made in the liver.

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ATTEMPTS TO CONTROL PARATHYROID TETANY BY THE ORAL ADMINISTRATION OF AMMONIUM CHLORIDE

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Several favorable reports have been made on the use of ammonium chloride by mouth in infantile tetany. (Freudenberg and György, 1922; Gamble and Ross, 1923; Anderson and Graham, 1924.) The same salt has been given intravenously in gastric tetany (Youmans and Greene, 1925.) In view of the results described, it seemed worth while to test its efficacy in controlling the effects of parathyroid removal.

It was shown by Haldane (1921) that ammonium chloride produces acidosis. Anderson and Graham (1924) reported that acidosis, produced in several different ways, brought about in all cases a relief of the symptoms of infantile tetany. Wilson, Stearns and Janney (1915) noted that hydrochloric acid given intravenously would relieve the acute attacks of tetany in dogs after parathyroid removal. The animals did not recover permanently under such treatment, and the acid was not well tolerated by mouth. Sodium bicarbonate appeared to aggravate tetany.

It seemed to us likely that ammonium chloride by mouth might give a more prolonged and evenly-sustained acid effect than could be obtained from intravenous injections of hydrochloric acid. Several effective methods for controlling parathyroid tetany are already available (Luckhardt and Rosenbloom, 1922; Luckhardt and Goldberg, 1923; Dragstedt and Peacock, 1923; Collip, 1925). A study of the effects of ammonium chloride, however, might throw more light on a possible influence of the acid-base balance in determining the severity of symptoms after parathyroid removal.

Procedure. 1. *Diet.* Eight dogs, of which four were controls, were kept on a diet of white bread and milk. The bread was given ad libitum, the milk in rations of 30 to 50 cc. per kilogram of body weight daily. The diet was started two days before removal of the parathyroids, and was continued throughout the period of survival of the animal, except as noted below. Three other dogs, on ammonium chloride treatment, received a diet of white bread and raw beef liver. No controls were run on this diet. From the reports of others, however, (Dragstedt and Peacock, 1923) it

seems fairly certain that dogs so fed would be more susceptible to tetany than those kept on bread and milk. We cannot say that the diets were quantitatively constant. After parathyroidectomy the appetite becomes irregular, and in our experience dogs will frequently leave their food untouched, particularly after the onset of severe symptoms. Liquid food may be given by stomach tube, but it is by no means certain to be retained.

The bread and milk diet may be criticised on the ground that the calcium in the milk is sufficient to reduce the severity of tetany, if not to prevent it altogether. Dragstedt and Peacock (1923) controlled tetany and had dogs survive indefinitely on a diet of skim milk, white bread and lactose. It appears from their experiments, however, that the added lactose was an important factor in this diet. The same point is very clearly shown in the work of Inouye (1924). Salvesen (1923), it is true, had dogs recover completely on a diet of milk and bread only, the milk ration being approximately the same as in our experiments. Five out of ten of his dogs, however, died within four days after parathyroidectomy. Of the remaining five, three received daily calcium injections, so that only two survivals can be definitely attributed to the dietary regime. From the literature it seems to be a fair conclusion that the majority of adult dogs, kept on bread and milk with no other treatment, will die within two weeks after removal of the parathyroids. None of our control animals survived longer than eleven days (see table 1).

2. *Dosage and method of administration.* As a rule, the ammonium chloride was given in water solution, by stomach tube. A 2.0 per cent solution was used at first, but vomiting followed so often that the concentration was reduced to 1.0 per cent. Even then vomiting was not infrequent, and seemed to occur more readily after parathyroidectomy than in normal dogs. For this reason, it was practically impossible to control the dosage accurately. Only one dog of the entire series (no. 8) retained every dose of the ammonium chloride. In general, prolonged medication seemed to aggravate the tendency to vomit. When this occurred, the daily dosage had to be reduced, to lessen the irritant effect of the salt. The amount given averaged about 0.5 gram per kilogram of body weight per day. The extremes were 1.0 gram (dog 1) and 0.25 gram (dog 11).

The records of three operated dogs were discarded. One of them, intended as a control, died on the second day after parathyroidectomy. He had appeared normal previously, but the condition of the eyes and nostrils a few hours after the operation made us fear that a beginning case of distemper had been overlooked. Two other dogs, kept on ammonium chloride with bread and milk, did not develop tetany at all. The ammonium chloride was stopped on the sixth day, and on the ninth day raw ground meat was substituted for the milk in the diet. No tetany developed during a further observation period of three weeks. It was con-

cluded that the animals probably had accessory parathyroid tissue sufficient for complete protection against tetany. This is made more probable by the fact that blood calcium determinations made on one of the dogs showed normal values during the post-operative period (9.2 and 9.7 mgm. on the third and sixth days, respectively). Unfortunately, no blood calcium analyses were made on the second dog.

Results. We have included in the series below only animals which showed tetany at some time. Ammonium chloride was not given to three dogs until after the first attack of tetany. In the remaining cases dosage was begun on the day of operation, but where tetany failed to develop during treatment the ammonium chloride was withdrawn until after tetany appeared.

Dogs 4 and 5 each received an intravenous injection of 2.0 grams of calcium lactate during severe attacks on the second and fifth days, respectively, after operation. A similar injection was given to dog 6 of the control series on the sixth day. Dog 8 received 2.0 grams to relieve severe tetany induced on the 23rd post-operative day by sodium bicarbonate (see protocol). With these exceptions, no calcium was given to any of the animals.

Table 1 gives a brief history of the entire series.

The principal points brought out in the above table are first, that animals on bread and meat diet, with ammonium chloride, survive on the average longer than those on bread and milk diet without it. So far as diet alone is concerned, the former group should have succumbed the more quickly after loss of the parathyroids. Second, as between two groups receiving the same diet, those animals treated with ammonium chloride survived much longer than the controls. The relation between the treatment and the course of tetany can be seen better, perhaps, from the following abbreviated protocols:

Dog 1. Male, 12.0 kilograms. Diet, white bread ad libitum, one-half pound raw beef liver.

October 21, 1924. Diet begun.

October 23. Thyroids and parathyroids removed.

October 25. Severe tetany; 2.0 grams calcium lactate intravenously. Later, 200 cc. 2 per cent ammonium chloride by mouth at 3-hour intervals, 12 grams in all.

October 26. 12 grams NH_4Cl, divided into three doses. One lost by vomiting. No tetany.

October 27. Same dosage. Vomiting twice. No tetany.

October 28. No NH_4Cl retained. Moderate tetany all day.

October 29. Retained 4 grams only. No tetany.

October 30. 7.0 grams. Dog in good condition.

October 31 to November 6. Dosage gradually reduced to 3 grams daily, which was retained. No tetany at any time. The appetite was good and the dog appeared normal.

November 7–16. Dosage the same. No tetany. The appetite was gradually lost, however, and the animal became thin.

TABLE 1

DOG	DATE OF OPERATION	FIRST TETANY	SURVIVAL, DAYS	REMARKS
A. Controls. Diet, bread and milk				
6 ♂	April 15	April 17	11	Tetany attacks on 7 days of the survival period. 2.0 grams calcium lactate given intravenously during severe tetany April 20
7 ♂	April 7	April 8	5	Severe tetany April 8, 10 and 11
9 ♂	May 23	May 24	7	Only two attacks of tetany
10 ♂	May 23	May 25	7	Tetany once only. Depressed during remainder of period
B. Animals on diet of bread and raw liver				
1 ♂	October 23	October 25	26	Ammonuim chloride not given until after first tetany (see protocol)
3 ♂	November 8	November 16	20	Medication begun day of operation. Discontinued November 14, until after first tetany (see protocol)
4 ♂	November 21	November 23	10	No ammonium chloride until after first tetany. 2.0 grams calcium lactate intravenously November 23. Severe tetany November 23 and 24. None thereafter
C. Animals on bread and milk diet, with ammonium chloride				
2 ♀	November 7	November 9	26	Ammonium chloride from day of operation. No tetany after first attack, except slight twitching of scalp and neck muscles on November 20
5 ♀	March 4	March 6	27	No ammonium chloride until after first tetany. 2.0 grams calcium lactate intravenously March 6. Severe tetany March 6, 9, 14 and 21, with several mild attacks in the intervals. None after March 22
8 ♂	May 2	May 5	197	Ammonium chloride from day of operation (see protocol)
11 ♂	June 5	June 7	39	2.0 grams ammonium chloride June 6. No more until after first tetany. Severe attacks June 9 and 11, but none later. Dog received ammonium chloride regularly after June 7

November 17. Nose and eyes purulent, as in distemper. Ammonium chloride withheld, in the hope that the dog might regain his appetite, but he did not.

November 18–19. Depressed and hardly able to walk. Found dead on the morning of the 20th.

Dog 3. Male, 8.5 kilograms. Diet, bread and raw liver.

November 6, 1924. Placed on diet.

November 8. Thyroids and parathyroids removed.

November 9. 8.0 grams NH_4Cl, of which part was lost by vomiting. No symptoms.

November 10–14. 6 grams daily. No tetany.

November 15. No NH_4Cl. No tetany.

November 16. Tetany, moderately severe. 4 grams NH_4Cl.

November 17. Mild tetany. 6 grams.

November 18–26. 4 to 6 grams daily. No more tetany, but the appetite was gradually lost and the dog became more and more depressed.

November 27. No NH_4Cl given. Dog refused food.

November 28. Died.

The loss of appetite shown by dog 1 might be attributed to the distemper which developed during the fourth week after the operation. Dog 3, however, showed almost as complete a loss of appetite, without distemper. We felt that the evident irritation of the gastro-intestinal tract by ammonium chloride was probably the chief cause of the persistent anorexia. Accordingly a smaller initial dosage was used in the later experiments.

Dog 8. Male, 15 kilograms. Diet, bread and milk (500 cc. daily).

April 30, 1925. Diet begun.

May 1. 6 grams NH_4Cl.

May 2. Thyroids and parathyroids removed.

May 3. 4 grams NH_4Cl. No symptoms.

May 4. No medication. No symptoms.

May 5. Moderate tetany. 8 grams NH_4Cl.

May 6–8. 5 grams daily. No tetany.

May 9. 5 grams. Twitching of scalp muscles.

May 10. No medication. No tetany.

May 11. Moderate tetany. Later, 5 grams NH_4Cl.

May 12. No medication. No tetany.

May 13. Tetany, more severe. 8 grams NH_4Cl.

May 14. Stiffness in hind legs. 4 grams.

May 15. Dog normal. 5 grams.

May 16. No medication. Still in good condition.

May 17. Moderate tetany. 4 grams NH_4Cl.

May 18–24. 4 grams daily. Dog in good condition, eats well, no tetany.

May 25. Dog normal in the morning. At 9:30, gave 15 grams $NaHCO_3$ by stomach tube in 400 cc. water. Slight tetany appeared about 12:30 p.m., growing severe during the afternoon. Gave 2 grams of calcium lactate intravenously, and later 4 grams NH_4Cl per os.

May 26–27. 4 grams NH_4Cl. Dog normal.

May 28. No medication. No tetany.

May 29. Slight tetany. 4 grams NH_4Cl.

May 30–June 2. No medication. Dog normal.

June 2. Diet changed. One pint milk, one pound raw hamburger.

June 3–4. No medication. No tetany.

June 5. Moderate tetany all day. Resumed NH_4Cl, 2 grams daily.

June 6–18. Same diet and dosage of NH_4Cl. Dog in excellent condition. No tetany.

June 19. No more ammonium chloride given. Dog placed with stock animals, on mixed diet, chiefly table scraps.

June 19 to November 14, the dog remained in good health and was never seen in tetany. He was not under careful observation during the entire period, however, so it is possible that some attacks may have occurred.

November 15. Found dead in the morning, having been apparently in good condition the previous day.

The effect of ammonium chloride administration on the blood calcium level. This has been studied in cases of infantile tetany by Gamble and Ross (1923) and by Anderson and Graham (1924). No constant effect was found. We have attempted to follow the curve of total calcium concentration in the blood for periods of five to six hours after the administration of a single large dose of ammonium chloride.

Determinations were made by a modification of the Kramer and Tisdall method permitting the use of oxalated whole blood. This modification was worked out by one of us (W. C. A.) and will be described in a later publication. Calcium values found by it agree with those obtained by the original Kramer and Tisdall method, within the limits of error of the latter. Calcium added to blood samples is also recovered quantitatively, with as much accuracy as the older method gives.

Blood was drawn from the saphenous vein at intervals of one to two hours after a single dose of 0.5 gram of ammonium chloride per kilogram body weight. In some experiments the carbon dioxide capacity was determined at the same intervals by the method of Van Slyke, the whole blood being used. The experiments were started at least twelve hours after feeding.

The ammonium chloride was given in 2 per cent solution, by stomach tube. Many experiments were interrupted by the vomiting of the entire dose.

Our findings confirm the earlier work in that no consistent rise in total calcium followed the giving of ammonium chloride. In only two experiments out of twelve did we observe an unmistakable rise. One showed an increase from an initial figure of 8.4 to 12.1 mgm., another from 8.2 to 13.1 mgm. Ca per 100 cc. of whole blood. For the remaining experiments the maximum variation above the initial blood calcium figure seen in any case was 1.9 mgm. The maximum variation below the initial value was 1.5 mgm. Changes of this order can hardly be considered significant. Some fluctuation in the blood calcium level probably occurs normally, and variations up to approximately 0.3 mgm. may be due to the limitations in accuracy of the method used.

There is no adequate evidence, then, that ammonium chloride acts through increasing the blood calcium concentration. Gamble and Ross, in the paper already mentioned, expressed the belief that an increase in ionized calcium did occur, and that the alleviation of symptoms was due to it. There seems to be no direct evidence for such an increase in ionized calcium, although it would on theoretical grounds be expected to occur. We have nothing to offer on the point.

Discussion. The results appear to show that ammonium chloride reduces the frequency and severity of tetany attacks in parathyroidectomized dogs. The period of survival is prolonged, but complete recovery does not as a rule occur. With the exception of dog 8, all the animals passed through a period of depression, anorexia, and progressive emaciation before death. Because of this anorexia, the average daily food intake was probably less than that of the controls. Therefore any difference which may have existed in the daily calcium supply was such as to favor the control group.

The beneficial effects of ammonium chloride, while definite, are less marked than one might perhaps have been led to expect from the reports cited of its use in infantile tetany. We can readily believe, however, that it might prevent tetany altogether in cases where only a partial deficiency of the parathyroids existed. We are inclined to think that the anorexia and depression, mentioned above, were largely due to the ammonium chloride itself, and that smaller doses, used throughout, might have been followed by longer average survival. This will be determined by further experiments.

Our results may be construed in such a way as to fit almost any of the well known theories as to the cause of parathyroid tetany. We wish merely to say that further evidence has been given that acid-producing substances tend to relieve the condition. Whether this is due to the acidosis *per se*, or to a concomitant effect on the blood calcium, cannot be stated at present. It is true that we have repeatedly induced tetany, when it had been absent for several days, by giving an alkaline salt, sodium bicarbonate. (See protocol, dog 8, for one such instance.) In animals which have survived parathyroidectomy for a period of five or six weeks, however, we have found it difficult or impossible to induce tetany by similar doses of sodium bicarbonate. The effect of the basic salt may be due to an influence on the blood calcium, in a direction opposite to that of ammonium chloride. Or, animals after parathyroidectomy may for a time be peculiarly susceptible to the "sodium poisoning" described by Isidor Greenwald (1921). Tetany is not induced by even larger doses of sodium bicarbonate in dogs with parathyroids intact (Boyd, 1925).

SUMMARY

1. Ammonium chloride, given by mouth to parathyroidectomized dogs in doses of 0.25 to 1.0 gram per kilogram of body weight daily, reduces the frequency and severity of tetany attacks.

2. The period of survival of such animals is considerably prolonged, but recovery does not as a rule occur. Death is commonly preceded by a period of several days of anorexia and depression, without tetany.

3. We have not been able to obtain a consistent rise in the blood calcium concentration after the administration of ammonium chloride.

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BASAL METABOLISM IN PROLONGED FASTING IN MAN

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Mr. Frederick Hoelzel, the subject of the present study, fasted a number of times in connection with experiments he performed on himself while investigating the conditions affecting the acidity of gastric juice. Early last summer he fasted for 33 days. A month later he decided to undergo another period of fasting of at least 40 days' duration. At the request of Doctor Carlson I made a series of basal metabolism determinations on Mr. H. both during the fast and for four months after he resumed eating.

The work of Benedict and his co-workers and that of Kunde (1923) on the effects of prolonged fasting on metabolism have been discussed by Boothby and Sandiford in a review article on basal metabolism (1924). Their results will be referred to later on. Labbe and Stevenin (1922) and in another communication Labbe, Stevenin and Nepteux (1922) reported a case of an almost absolute voluntary fast undergone by a man 42 years of age. The man fasted for 42 days. He took no water on certain days, while on others he drank some water, or lemonade, or beer. The basal metabolism was less than half the normal value at the end of the fast. The prefasting basal metabolism was 1040 cal. per sq. m. of body surface per 24 hours, and it fell to 520 at the end of the fasting period. They followed the subject's basal metabolism for only 29 days after he resumed eating, and at the end of that time it was 948 cal., or not quite up to the normal level. The results were discussed in greater detail in a recent paper by Labbe and Stevenin (1925), where they analyzed their findings in the light of the figures given by Benedict and by Kunde. Quite recently Takahira (1925) obtained exhaustive data on five men, who fasted for 12, 16, 17, 26 and 30 days respectively. He made only one or two determinations of basal metabolism before the beginning of the fast in each case and followed the basal metabolic rate for a few days after the fast. I shall discuss the results of Labbe and Stevenin and those of Takahira in connection with my own findings on Mr. H.

The indirect method was employed in the determination of the basal metabolic rate. The Sanborn-Benedict apparatus was employed. Mr. H. was accustomed to breathing through the mouthpiece of the apparatus, as he served as a subject for Kunde (1923). He was allowed to lie down

and relax for 30 minutes before each determination. He had to make a long trip in the street-car from his home to the laboratory, and for this reason his basal metabolic rate was measured only three times a week during the fasting period and rarer in the four-month period following fasting. The heat production was calculated on the basis of oxygen consumption alone and it was considered that the R. Q. was 0.82 in the control periods, and 0.70 during fasting. Some of the figures given here are based on only one determination, but in many cases as many as six determinations were made consecutively. This was done especially for the purpose of studying the degree of variability of the basal metabolic rate in the course of one or two hours in the morning. On several occasions a narrow rubber tube was passed through the mouthpiece, and this tube connected a thin rubber balloon in the subject's stomach with a water manometer. Thus it was possible to record the motor activity of the stomach during the basal metabolism determinations. The tests were generally made early in the morning, 14 hours after the last meal, each test lasting 10 minutes.

As already indicated, this fast was undertaken one month after the completion of a 33-day fasting experiment. The subject was gaining in weight throughout the intervening period. Unlike Labbe's subject, he partook of no nutritive material such as lemonade or beer during the entire fast, from 10 p.m. on August 2, 1925, till 3:30 p.m. on September 13, nearly 42 days. This is probably the longest absolute fast ever studied in man. Mr. H. did, however, take some non-nutritive material during the fast, partly to see if it had any effect on basal metabolism, partly in connection with his own research. Beginning on August 19 and up to August 24 he ate a certain quantity of talcum powder daily. The mixture he used was made up of one part petroleum jelly to three parts of talcum, and in five days a total of 740 grams of this mixture was ingested. On August 24 he replaced the talcum powder by cellulose ("cellu-flour"), also mixed with petroleum jelly, and in the course of 19 days 1450 grams of this preparation were taken. About 500 mgm. of saccharin were used to flavor the talcum powder and the cellulose eaten during the fast. Moderate amounts of water were taken at all times—merely to quench thirst. On September 9, 10, 11 and 12 enemas were administered, and this may account for the comparatively greater loss in body weight during the last four days of the fast (table 2). Besides minor activities of all kinds, Mr. H. walked from $1\frac{1}{2}$ to 8 miles daily throughout the fast.

The results obtained are given in table 1 and in figure 1. Five determinations made before fasting showed a basal metabolism varying from 1445 to 1570 cal. per 24 hours. On the fifth day of fasting the basal metabolic rate was higher than normal, but thereafter it gradually declined, and with minor fluctuations reached its lowest point on the last day of the fast, when it was only 978 cal. per 24 hours. The body weight decreased

very steadily during the fast, the daily loss becoming progressively smaller (table 2). At the beginning of the fast the weight was 65.5 kgm., and it fell to 48.9 kgm., a loss of 25 per cent of the original weight. The basal metabolism for the same period fell from an average prefasting level of 1517 cal. per 24 hours to 978, a decrease of 36 per cent. The decrease in

TABLE 1

Basal metabolic rate before, during and after 41-day fast

DATE	CALORIES PER			PULSE	DATE	CALORIES PER			PULSE
	24 hours	Kilogram	Square meter			24 hours	Kilogram	Square meter	
	Pre-fasting period				September				
1925					25	1310	23.7	820	78
July					29	1466	25.1	899	72
23	1570	25.1	940	80	October				
25	1549	24.9	927	78	14	1570	24.3	923	
27	1445	22.9	860		16	1530	23.7	905	82
29	1501	23.6	893	68	20	1369	21.4	810	72
31	1522	23.8	901		22	1437	22.6	850	72
	Fasting period				24	1570	24.2	923	72
August					27	1516	23.4	892	72
5	1545	24.6	920	76	31	1440	22.6	852	64
7	1666	28.5	998	68	November				
10	1343	22.5	819	58	4	1355	21.2	806	64
12	1282	21.7	786	53	7	1534	24.5	913	63
14	1275	21.9	787	54	14	1739	27.7	1035	58
17	1177	20.4	725	51	21	1914	29.9	1133	68
19	1177	20.6	727	47	28	1888	29.7	1117	67
21	1177	20.8	729	54	December				
24	1157	20.7	723	50	5	1625	26.1	967	70
26	1089	19.7	686	50	12	1804	28.5	1074	73
28	1076	19.6	681	52	22	1633	25.9	972	63
31	1095	20.5	702	50	23	1644	26.2	979	58
September					1926				
2	1120	21.1	718	57	January				
5	1089	20.8	703	57	16	1664	26.5	990	62
8	1034	20.1	672	54	30	1642	26.3	977	68
11	978	19.5	639	54	February				
	Post-fasting period				13	1442	23.1	863	64
15	1110	22.0	726	60	16	1519	24.3	904	61
18	1306	25.1	830	74					
22	1285	23.8	819	75					

basal metabolism being much greater than that in body weight, it is evident that there was a decrease in heat production per unit of body weight. Indeed the normal average heat production per kilogram of body weight per 24 hours was 24.1 cal., and it fell to 19.5 cal., showing a decrease of 19 per cent. This decrease is even more striking when one accepts the square

meter of body surface instead of the kilogram of body weight as a unit of measurement. Thus the average prefasting heat production per square meter was 904 cal. and at the end of the fast it was only 639 cal., a decrease of 29 per cent. The heart rate fell at first almost as rapidly as the basal metabolism, and on the 17th day of fasting it reached its minimum of 47 beats per minute, the normal being around 70. It then picked up a little and continued to fluctuate between 50 and 57 till the end of the fast. When the subject resumed eating the basal metabolic rate began to rise

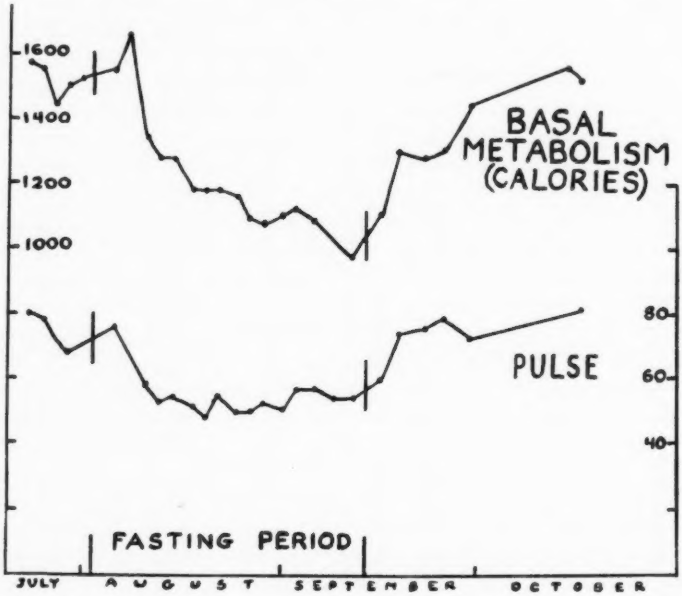

Fig. 1. The basal metabolism and the heart rate during and after a 42-day fast in man.

at once, and at the end of one month's time it was 1570, or within the normal prefasting variation.

The basal metabolism varied considerably during the second month of realimentation, but at no time did it exceed the highest figure of the prefasting period. On the contrary it was much lower than normal at times. Then the basal metabolism began to rise and far exceeded the normal value, but toward the end of the fourth month of realimentation it was down to normal again. To understand this sudden rise in basal metabolism which began two months after fasting, it is important to know the dietary of the subject, as an interesting parallel is revealed between

TABLE 2

Weight before, during and after 41-day fast

DATE	KILOGRAMS	DATE	KILOGRAMS	DATE	KILOGRAMS	DATE	KILOGRAMS
1925		September		October		December	
July		5	52.5	21	62.5	5	63.1
23	62.5	6	52.3	22	63.6	6	63.0
24	62.0	7	51.6	23	64.1	7	63.1
25	62.3	8	51.4	24	64.8	8	63.2
26	63.0	9	51.3§	25	62.7	9	63.4
27	63.0	10	50.2§	26	64.1	10	63.2
28	64.1	11	50.0§	27	64.8	11	63.4
29	63.6	12	49.1§	28	63.2	12	63.4
30	63.6	13	48.9‖	29	64.5	13	63.6
31	63.9	14	50.4	30	64.3	14	63.8
August		15	50.4	31	63.8	15	64.3
1	64.1	16	52.3	November		16	64.1
2	65.0*	17	52.6	1	62.7	17	63.5
3	65.5	18	52.0	2	63.0	18	63.3
4	63.6	19	54.0	3	63.2	19	62.7
5	63.0	20	52.7	4	63.4	20	63.2
6	62.3	21	53.9	5	62.5	21	63.4
7	61.8	22	53.9	6	62.0	22	63.0
8	61.0	23	54.6	7	62.7	23	62.7
9	60.2	24	55.7	8	61.8	24	62.5
10	59.6	25	55.2	9	61.4	25	61.9
11	59.1	26	56.1	10	62.7	26	62.3
12	59.1	27	58.6	11	62.7	27	63.2
13	58.4	28	58.0	12	63.2	28	63.2
14	58.2	29	58.4	13	62.7	29	63.1
15	57.8	30	58.6	14	62.7	30	63.5
16	57.5	October		15	63.4	31	64.3
17	57.3	1	59.3	16	63.6	1926	
18	57.0	2	59.8	17	63.2	January	
19	56.8†	3	60.7	18	63.6	1	63.2
20	56.4	4	61.1	19	63.2	2	63.0
21	56.1	5	61.4	20	63.3	3	63.2
22	56.1	6	62.7	21	64.0	4	63.5
23	55.9	7	62.5	22	63.8	5	64.3
24	55.9‡	8	63.4	23	63.7	6	64.9
25	55.5	9	63.4	24	63.6	7	64.0
26	55.2	10	63.8	25	64.0	8	63.4
27	54.8	11	63.6	26	64.1	9	63.4
28	54.8	12	63.5	27	64.0	10	63.6
29	54.1	13	63.8	28	63.6	11	64.6
30	53.7	14	64.6	29	63.6	12	64.0
31	53.4	15	64.3	30	63.8	13	64.0
September		16	64.5	December		14	63.6
1	53.4	17	63.8	1	64.1	15	63.4
2	53.2	18	64.1	2	64.9	16	62.7
3	52.8	19	63.6	3	64.7	17	63.0
4	52.7	20	64.1	4	63.8	18	63.2

* Last food 10:00 p.m.

† Began use of talcum preparation.

‡ Began use of cellulose in place of talcum preparation.

§ Enema.

‖ Resumed eating 3:30 p.m.

the total caloric value of the diet and especially the protein intake and the *basal* metabolic rate.

Mr. H. resumed eating on September 13, and he fed on fruits, vegetables, olive oil, butter, cream, sugar and other protein-poor foods up to September 29. Then a high protein diet had to be instituted, because of a marked post-fasting edema which was thought to have been aggravated by the foregoing low protein diet. During the next nine days he ate four pounds of meat daily on the average. On the following six days protein was taken in gradually decreasing amounts. On October 15 a low protein diet was instituted, and by October 20 the basal metabolism fell to 1369 cal. per 24 hours. Then a high protein diet was resorted to (36 to 48 egg whites daily), and the basal metabolism climbed up to 1570 cal. On November 2 protein restriction was reinstituted, and on November 4 the basal metabolism was low again, only 1355 cal. Then there followed a couple of modified fast days (250–600 cal.), and beginning with November 6 the diet was made rich in total calories (3100). On November 7 the basal metabolism rose to 1534. Up to this point the metabolism was lower rather than higher than the prefasting level (table 1). On November 10 the diet was made not only rich in protein (30–42 egg whites daily), but also high in total calories (2750–3850 cal. daily) and the combined effect of high protein and high total calories was a sudden increase in basal metabolism, which was 1739 cal. on November 14 and 1914 cal. on November 21. On November 24, continuing on a diet of an unusually high caloric value for a relatively inactive individual, 3200 to 3850 cal., but decreasing the protein to 20 grams, the basal metabolism was brought down to 1625 cal. on December 5, still higher than normal, but much lower than it was under a diet rich in protein and of high caloric value. On December 7 the subject began to increase his protein intake, starting with 8 to 9 ounces of meat per day, and gradually going up to 40 ounces on December 11. On December 12 his basal metabolism was high again, 1804 cal. Continuing the high protein, but gradually decreasing the total calories, he brought his basal metabolism down to 1633 cal. on December 22 and 1664 on December 23. This condition continued with minor modifications till February 1 when a diet of a moderate amount of protein and of a medium or low total caloric value was resorted to. As a result of this by the middle of February the basal metabolism was entirely normal again, 1442 cal. on February 13 and 1519 cal. on February 16. It seems then fairly conclusive that the caloric value of the diet and especially the quantity of protein taken has a decided positive influence on the basal metabolic rate.

Another phenomenon revealed in this study is the marked variability in the amount of oxygen consumed per 10 minutes, when several determinations were made in succession. It was as an exception rather than the rule that perfect checks were obtained in the spirometer readings. It was

noticed incidentally that higher figures for oxygen consumption were obtained, if the subject felt powerful hunger contractions during the test. A simultaneous record was then made of oxygen consumption and of the motor activity of the stomach by the balloon method. Some of the results obtained are given in table 3. On August 31 strong gastric contractions were followed by an increase in the oxygen consumption from 166 cc. to 250 cc. per minute. On September 11 a less marked increase in oxygen consumed accompanied increased motor activity of the stomach. On October 24 two series of three determinations each were run. After the first series 50 grams of a mixture of two parts cellulose and one part petroleum jelly was taken with the intention of abolishing the hunger contractions. It was found that the ingestion of cellulose does not uniformly produce motor quiescence of the stomach, and further data on this subject will be published in the near future. On October 24 and November 14 figures for oxygen consumption before and after cellulose show no appreciable differences. On the whole variations of from 2 to 5 per cent in successive determinations were found to be very common, and occasionally even 10 per cent differences were noticed, without any observable phenomenon to account for these fluctuations. At times, however, the amount of oxygen consumed in several succesive determinations did not vary even a fraction of 1 per cent.

The data collected during the forty-two-day fast demonstrate very clearly that as a result of fasting there is a marked decrease in the basal metabolic rate. In the case of Mr. H. the basal metabolism was lowered 36 per cent. The loss in weight for the same period amounting to 25 per cent of the original weight, the basal metabolism per kgm. was decreased 19 per cent. This decrease is even more prominent, if one accepts the square meter of body surface as the unit of measurement. Thus the basal metabolism per square meter decreased from 904 cal. to 639 cal., or 29 per cent. In table 4 I arranged the results obtained by Labbe and Stevenin in a fast of 41 days, and those of Takahira in five different subjects who fasted from 12 to 30 days, alongside of my own. It will be seen that they agree in every particular. Labbe and Stevenin's subject showed a much greater decrease in basal metabolism than did Mr. H. or any of Takahira's subjects. But in all these cases the fall in heat production per square meter of body surface is greater than that per kilogram of body weight. Benedict in a 30-day fast obtained similar results, even though the decrease was not nearly so great. One may consider, then, as established that after an initial rise of variable duration there is a decrease in basal metabolism during fasting, the decrease becoming more marked with the length of the fast, and that the decrease is evident no matter what one chooses as a unit of measurement. Benedict's contention that mass rather than surface should be used as criterion for measuring heat production, based as it was on the

supposition that the metabolic rate per unit of body weight did not change during fasting, seems untenable. The summary figures reported by Kunde (1923) who studied the basal metabolic rate of two human subjects

TABLE 3

Showing variations in basal metabolic rate as indicated by consecutive determinations

DATE (1925)	TEST BEGAN (a.m.)	CC. O_2 PER MINUTE	PULSE	REMARKS
August 31	8:28	160.0	45	Gastric motor quiescence
	9:07	161.0	49	Little gastric motility
	10:01	166.0	56	Strong gastric contractions with tetany period
	10:18	250.5	54	Gastric motor quiescence
	10:39	174.5	54	Gastric motor quiescence
September 11	8:30	145.0		Some mild hunger contractions
	8:45	145.0		Some mild hunger contractions
	9:30	166.5		Increasing hunger contractions
	9:45	161.5	54	Gastric tetany period followed by motor quiescence
October 24	8:38	221.0		Before taking cellulose. Gastric tetany period
	8:52	230.0		Gastric motor quiescence
	9:06	227.0	72	Gastric motor quiescence
	10:18	216.5		30 minutes after taking cellulose. Gastric motor quiescence
	10:34	221.5		Gastric motor quiescence
	10:49	229.0	72	Gastric motor quiescence
November 14	8:29	269.5		Before taking cellulose. Very little gastric motility
	8:44	250.5		More gastric motility
	8:59	250.0	58	Slight further increase in gastric motility
	10:06	233.0		30 minutes after taking cellulose. Little gastric motility
	10:20	240.0		A few gastric pangs felt
	10:33	239.0	60	Gastric motor quiescence
November 28	8:30	265.0		No cellulose throughout. Strong hunger contractions
	8:54	272.0		Gastric tetany period
	9:09	262.5	67	Gastric motor quiescence
	10:14	277.5		Gastric motor quiescence
	10:29	287.0		Gastric motor quiescence
	10:43	282.0	70	Gastric motor quiescence
December 12	8:53	252.0		30 minutes after taking cellulose. A few hunger contractions
	9:08	263.5		Increasing hunger contractions

TABLE 3—*Concluded*

DATE (1925)	TEST BEGAN (a.m.)	cc. O_2 PER MINUTE	PULSE	REMARKS
December 12	9:24	262.0	76	Gastric tetany period
	10:26	258.0		30 minutes after taking more cellulose. Some strong gastric contractions
	10:41	258.5		More strong gastric contractions
	10:56	264.0	70	Gastric motor quiescence
December 23	8:30	234.5		Before taking cellulose. Little gastric motility
	8:45	240.0		Little gastric motility
	9:00	235.0	58	Some increase in gastric motility
	10:10	247.5		3 minutes after cellulose was fed to the subject while otherwise resting. Strong gastric contractions
	10:23	246.5		Decrease in gastric motility
	10:38	246.5	62	Further decrease in gastric motility

TABLE 4

The effect of fasting on body weight, basal metabolism and basal heat production per unit of weight and of surface

OBSERVER	DAYS OF FASTING	BODY WEIGHT			BASAL METABOLISM—CALORIES IN 24 HOURS			CALORIES PER KILOGRAM IN 24 HOURS			CALORIES PER SQUARE METER IN 24 HOURS		
		Original	Final	Loss	Original	Final	Loss	Original	Final	Loss	Original	Final	Loss
		kgm.	*kgm.*	*per cent*			*per cent*			*per cent*			*per cent*
Labbe et al.....	41	62.8	46.0	27	1787	782	56	26.6	17.0	36	1039	521	49
Takahira I......	12	50.2	42.9	14	1410	1067	24	28.1	24.8	13	904	730	19
Takahira II.....	16	43.5	36.0	17	1278	917	28	29.4	25.4	14	929	722	22
Takahira III....	17	48.1	41.8	13	1322	928	30	27.5	22.2	19	905	679	25
Takahira IV....	26	77.0	64.7	16	1655	1122	32	21.5	17.4	20	940	688	27
Takahira V.....	30	57.9	45.5	21	1525	935	39	26.4	20.6	22	919	624	32
Kleitman........	42	65.5	48.9	25	1517	978	36	24.1	19.5	19	904	639	29

and three dogs, during periods of fasting, are in distinct contradiction with the results obtained by Labbe and Stevenin, by Takahira, and by myself. In all her subjects she found an increase in basal metabolism per kilogram of body weight during fasting. Kunde's treatment of her data was criticised by Boothby and Sandiford and by Labbe and Stevenin (1925), and the reader is referred to their papers for the details. Suffice it to say that she failed to calculate the basal heat production per square meter of body surface, even in the case of her human subjects, and that in reducing her figures to kilogram of body weight, she used an average weight

and an average basal metabolism for the entire fasting period, even though both weight and metabolism were constantly decreasing.

What is the cause of the lowering in basal metabolism that occurs during fasting? At present we can only make conjectures on this subject. Labbe and Stevenin, like many others, think that the body acquires new habits and that the diminution of metabolism is a means of resisting inanition. That, of course, merely restates the original question. It seems more probable that the thyroid gland suffers some reduction in activity in the course of the fast, and that this is responsible for the lowering of the basal metabolism. In this connection the facts cited by Jackson in his book on *Inanition* (1925) are of interest. It was found that the decrease in weight of the adult human thyroid in various conditions of malnutrition amounted to 20 to 47 per cent of the weight of the normal gland, and that in famine-stricken children the decrease was 45 to 70 per cent. Unfortunately, there is no information on the changes in thyroid gland in acute inanition in man, and the data on animals are somewhat conflicting.

Neither Benedict, nor Labbe and Stevenin, nor Takahira followed the basal metabolism of their subjects for more than three or four weeks after the conclusion of the fast. Labbe and Stevenin found that 29 days after realimentation began the weight returned to normal but the basal metabolism was only 1634 cal. as compared with 1787 before the fast, showing a subnormal metabolism. Takahira reports that in the 26-day fast the metabolism returned to normal in 8 days, and in the 30-day fast, in 21 days. Kunde studied the post-fasting metabolism for a much longer time, and she found that in the first two months the average basal metabolism per kilogram of body weight increased 6.1 and 2.1 per cent in her two human subjects, and that the per kilogram increase in the dogs was much more marked. Under the peculiar dietary conditions of this experiment my results fail to confirm Kunde's finding, in that in two months after the fast the basal metabolism was at no time higher than normal, either per unit of weight or per unit of surface. What decreases the value of my results is that the diet was not uniform enough, and that the fast I studied was undertaken only one month after the conclusion of a previous 33-day fast, which of itself might have increased the basal metabolism.

The variations in the diets followed by Mr. H. after the fast showed that the total calories and the amount of protein taken have a decided influence on basal metabolism. Lusk (1923) holds that basal metabolism corresponds with the minimal heat production 18 hours after taking a mixed diet. Our determinations were made 14 hours after the last meal, and the increased metabolism might be considered as a persistence of the specific dynamic action of the food ingested. It must be added that generally no protein food was taken after 3 p.m., and the basal metabolism determinations were made at 9 a.m., so that the basal metabolism was measured 18

hours after taking protein (at 6 p.m., some light supper poor in protein or containing no protein was eaten). As a rule basal metabolism is measured 12 to 14 hours after the last meal, and the character and size of the meal may have a decided influence on the figure obtained.

It was shown by Rubner on dogs (quoted from Lusk, 1923) that "if a large quantity of protein be ingested day after day, then the usual specific dynamic action occurs and also a continued 'secondary' rise in total day-to-day metabolism, which increases with the continual increase in protein metabolism." The data obtained on Mr. H. would tend to show that this secondary rise can also be observed in man, in basal metabolism, and that it depends not only on the protein intake, but also on the total caloric value of the diet.

Finally the variations in metabolism from day to day and from hour to hour were a prominent feature. Benedict and Crofts (1925) observed similar variations in consecutive determinations of basal metabolism in man. These variations generally do not exceed 2 or 3 per cent, but they may be as high as 10 per cent. What they are due to is unknown at present. Hunger contractions of the stomach may or may not affect basal metabolism. It probably depends upon whether the hunger pangs are painful enough to cause a general stiffening of the body musculature. Doctor Carlson thinks that the fluctuations in thyroid activity from day to day, and even from hour to hour may be responsible for the variations in basal metabolism. The variations exist, however, and, especially when one recalls that basal metabolism is higher in the afternoon than in the morning, it seems erroneous to calculate basal heat production for 24 hours on the basis of one or two ten-minute periods of observation. It would be much better to say that so many calories were produced in 10 minutes during the morning, bearing in mind the possible error of 5 per cent and the probable influence of the meals taken the previous day.

SUMMARY

1. After a short initial rise there is a marked decrease in basal metabolism in man as a result of fasting, both total and per unit of weight and of surface.

2. Under the conditions of this experiment there was no definite rise in metabolism during a two-month period after fasting.

3. The total caloric value of the diet and the protein intake may have a decided positive effect on the basal metabolic rate.

4. There are spontaneous variations of as much as 5 per cent in the figures for basal metabolism obtained in several consecutive 10 minute tests.

I am greatly indebted to Mr. Frederick Hoelzel for the splendid way in which he coöperated in this study and for the preparation of some of the tables. I also wish to thank Miss Erma Smith who made a number of the basal metabolism determinations.

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PHYSIOLOGICAL REVIEWS

PURPOSES

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Contents of Volume V, 1925

Contents of Volume VI, 1926